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Australian Government

Patent Office
Canberra

I, JULIE BILLINGSLEY, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2003901579 for a patent by MONASH UNIVERSITY as filed on 07 April 2003.

I further certify that the above application is now proceeding in the name of CORTICAL PTY LTD pursuant to the provisions of Section 113 of the Patents Act 1990.

PRIORITY DOCUMENT

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WITNESS my hand this
Twenty-seventh day of April 2004

J. Billingsley

JULIE BILLINGSLEY
TEAM LEADER EXAMINATION
SUPPORT AND SALES

Regulation 3.2

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Therapeutic Molecules and Methods-3"

The invention is described in the following statement:

THERAPEUTIC MOLECULES AND METHODS – 3

FIELD OF THE INVENTION

The present invention relates generally to the treatment of diseases or conditions resulting from cellular activation, such as inflammatory or cancerous diseases or conditions. In particular, the invention relates to the use of phenyl substituted cyclic derivatives to inhibit the cytokine or biological activity of macrophage migration inhibitory factor (MIF), and diseases or conditions wherein MIF cytokine or biological activity is implicated.

BACKGROUND OF THE INVENTION

MIF is the first identified T-cell-derived soluble lymphokine. MIF was first described as a soluble factor with the ability to modify the migration of macrophages (1). The molecule responsible for the biological actions ascribed to MIF was identified and cloned in 1989, (2). Initially found to activate macrophages at inflammatory sites, it has been shown to possess pluripotential actions in the immune system. MIF has been shown to be expressed in human diseases which include inflammation, injury, ischaemia or malignancy. MIF also has a unique relationship with glucocorticoids by overriding their anti-inflammatory effects.

Recent studies have indicated that monoclonal antibody antagonism of MIF may be useful in the treatment of sepsis, certain types of cancers and delayed type hypersensitivity. Antibody antagonism of MIF has also been shown to have activity in adjuvant- or collagen-induced arthritis animal models and other models of inflammatory and immune diseases.

Although antibody antagonism of MIF is one potential way to provide therapeutic treatments, such biological molecules can be expensive to prepare on a commercial basis and further, can be limited in the way they are administered (generally by injection) and do not readily lend themselves to formulations for administration by other means eg oral administration.

Small molecule inhibitors may overcome one or more such difficulties connected with the

use of biological therapeutic treatments. There exists a need, therefore, for small molecule inhibitors of the cytokine or biological activity of MIF. Small molecule inhibitors of the cytokine or biological activity of MIF would have therapeutic effects in a broad range of diseases, whether given alone or in combination with other therapies.

Further, glucocorticoids have been used to treat human diseases for over fifty years and are effective in a range of diseases which include inflammation, injury, ischaemia or malignancy. Although debate continues in relation to their impact on disease progression, their influence on symptoms and signs of inflammation, especially in the short term, can be dramatic.

Despite their benefits and efficacy, the use of glucocorticoids is limited by universal, predictable, dose-dependent toxicity. Mimicking Cushing's disease, a disease wherein the adrenal glands produce excess endogenous glucocorticoids, glucocorticoid treatment is associated with side effects including immunosuppression (resulting in increased susceptibility to infections), weight gain, change in body habitus, hypertension, oedema, diabetes mellitus, cataracts, osteoporosis, poor wound healing, thinning of the skin, vascular fragility, hirsutism and other features of masculinization (in females). In children, growth retardation is also noted. These side effects are known as Cushingoid side effects.

Since the side effects of glucocorticoids are dose dependent, attempts to reduce the dosage requirement have been investigated, including combination therapies in which glucocorticoids are administered with other therapeutic agents. These combination therapies are sometimes referred to as "steroid-sparing" therapies. However, currently available combination therapies are non-specific as the other therapeutic agents do not address biological events which inhibit the effectiveness of glucocorticoids. Such combination therapies are also typically associated with serious side effects.

Furthermore, glucocorticoids are incompletely effective in a number of disease settings, leading to the concept of "steroid-resistant" diseases. Agents which amplify or enhance the effects of glucocorticoids would not only allow the reduction of dose of these agents but may also potentially render "steroid-resistant" diseases steroid-sensitive.

There is a need for effective therapies which enable a reduction in the dosage level of glucocorticoids. There is also a need for effective treatment of "steroid-resistant" diseases. Preferably, such therapies or treatments would address factors which directly limit the effectiveness of glucocorticoids.

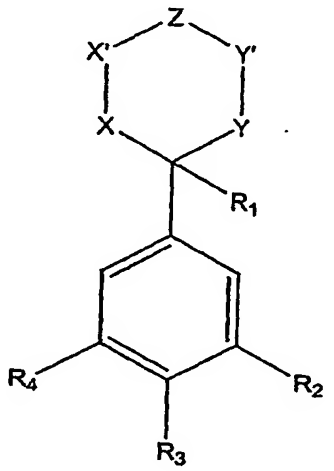
Therapeutic antagonism of MIF may provide "steroid-sparing" effects or be therapeutic in "steroid-resistant" diseases. Unlike other pro-inflammatory molecules, such as cytokines, the expression and/or release of MIF can be induced by glucocorticoids (3), (4). Moreover, MIF is able to directly antagonize the effects of glucocorticoids. This has been shown to be the case for macrophage TNF, IL-1β, IL-6 and IL-8 secretion (5), (6), and for T cell proliferation and IL-2 release (7). *In vivo*, MIF exerts a powerful glucocorticoid-antagonist effect in models including endotoxic shock and experimental arthritis (5), (8). In the context of an inflammatory or other disease treated with glucocorticoids, then, MIF is expressed but exerts an effect which prevents the glucocorticoid inhibition of inflammation. It can therefore be proposed that therapeutic antagonism of MIF would remove MIF's role in inhibiting the anti-inflammatory effect of glucocorticoids, thereby allowing glucocorticoids to prevail. This would be the first example of true "steroid-sparing" therapy. In support of this hypothesis is the observation that anti-MIF antibody therapy reverses the effect of adrenalectomy in rat adjuvant arthritis (9). By neutralizing the natural glucocorticoid 'counter-regulator' effect of MIF, it is envisioned that with MIF antagonism, steroid dosages could be reduced or even eliminated in inflammatory disease, particularly in those diseases that are associated with the glucocorticoid resistance (10), (11). There is a need, therefore, for therapeutic antagonists of the cytokine or biological activity of MIF.

SUMMARY OF THE INVENTION

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

In a first aspect, the present invention provides a method of inhibiting cytokine or biological activity of MIF comprising contacting MIF with a cytokine or biological activity

inhibiting effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof



(I)

wherein X and X' are independently selected from $-C(R_5)_2-$, –O-, -S-, $-N(R_5)-$, or taken together form $-C(R_5)=C(R_5)-$, $-C(R_5)=N-$, $-N=C(R_5)$, $-N(R_5)-N(R_5)-$ or –N=N-;

Y and Y' are independently selected from $-C(R_5)_2-$, –O-, -S-, $-N(R_5)-$, or taken together form $-C(R_5)=C(R_5)-$, $-C(R_5)=N-$, $-N=C(R_5)$, $-N(R_5)-N(R_5)-$ or –N=N-;

Z is $-C(R_5)_2-$, -O-, -S- or $-N(R_5)-$, or forms a covalent single or double bond between X' and Y', or Z together with X' or Y' forms $-C(R_5)=C(R_5)-$, $-C(R_5)=N-$, $-N=C(R_5)$, $-N(R_5)-N(R_5)-$ or –N=N-;

wherein when Z is –O-, -S- or $-N(R_5)-$, X' and Y' are $-C(R_5)_2$;

when X is –O-, -S- or $-N(R_5)-$, X' is $-C(R_5)_2$;

when Y is –O-, -S- or $-N(R_5)-$, Y' is $-C(R_5)_2$; or

X or Y together with the carbon atom bearing the phenyl group form a double bond;

R_1 is selected from hydrogen, $C_{1\text{-}20}$alkyl, $C_{2\text{-}20}$alkenyl, $C_{2\text{-}20}$alkynyl, $(A)_nC(O)R_6$, $(A)_nC(S)R_6$, $(A)_nS(O)R_6$, $(A)_nS(O)_2R_6$, $(A)_nOR_7$, $(A)_nSR_7$, $(A)_nN(R_8)$, $(A)_nC(=NR_9)R_{10}$ and $(A)_nR_{11}$, or when X or Y together with the carbon atom bearing the phenyl group form a double bond, R_1 is absent;

R_2 and R_4 are independently selected from hydrogen, C_{1-3}alkyl and $(A)_mR_{12}$;

R_3 is selected from C_{1-3}alkyl, $(A)_mR_{12}$, $(A)_m$aryl and $(A)_m$heterocyclyl;

R_5 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_nC(O)R_6$, $(A)_nC(S)R_6$, $(A)_nS(O)R_6$, $(A)_nS(O)_2R_6$, $(A)_nOR_7$, $(A)_nSR_7$, $(A)_pN(R_8)$, $(A)_nC(=NR_9)R_{10}$ and $(A)_nR_{11}$;

R_6 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, OH, OC_{1-10}alkyl, OC_{2-10}alkenyl, OC_{2-10}alkynyl, $O(A)_qR_{11}$, SH, SC_{1-10}alkyl, SC_{2-10}alkenyl, SC_{2-10}alkynyl, $S(A)_qR_{11}$, $N(R_{13})_2$, $[NH\text{-}CH(R_{14})C(O)]_s\text{-}OH$, $[NH\text{-}CH(R_{14})C(O)]_s\text{-}OC_{1-3}$alkyl, $[sugar]_s$ and $(A)_qR_{11}$;

R_7 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_qR_{11}$, C(O)H, $C(O)C_{1-10}$alkyl, $C(O)C_{2-10}$alkenyl, $C(O)C_{2-10}$alkynyl, C(O)-aryl, $C(O)(A)_qR_{11}$, $C(O)_2H$, $C(O)_2C_{1-10}$alkyl, $C(O)_2C_{2-10}$alkenyl, $C(O)_2C_{2-10}$alkynyl, $C(O)_2$-aryl, $C(O)_2(A)_qR_{11}$, C(S)H, $C(S)C_{1-10}$alkyl, $C(S)C_{2-10}$alkenyl, $C(S)C_{2-10}$alkynyl, C(S)-aryl, $C(S)(A)_qR_{11}$, C(S)OH, $C(S)OC_{1-10}$alkyl, $C(S)OC_{2-10}$alkenyl, $C(S)OC_{2-10}$alkynyl, C(S)O-aryl, $C(S)O(A)_qR_{11}$, $S(O)_tH$, $S(O)_tC_{1-10}$alkyl, $S(O)_tC_{2-10}$alkenyl, $S(O)_tC_{2-10}$alkynyl, $S(O)_t$-aryl, $S(O)_t(A)_qR_{11}$, $[C(O)CH(R_{14})NH]_s\text{-}H$, $[C(O)CH(R_{14})NH]_s\text{-}C_{1-10}$alkyl, $[C(O)CH(R_{14})NH]_s\text{-}C_{2-10}$alkenyl, $[C(O)CH(R_{14})NH]_s\text{-}C_{2-10}$alkynyl, $[C(O)CH(R_{14})NH]_s$-aryl, $[C(O)CH(R_{14})NH]_s\text{-}(A)_qR_{11}$ and $[sugar]_s$;

Each R_8 is independently selected from R_7 and $NHC(=NR_{15})NH_2$;

R_9 is selected from hydrogen and C_{1-6}alkyl;

R_{10} is selected from C_{1-6}alkyl, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, OH, OC_{1-3}alkyl, SH and SC_{1-3}alkyl;

R_{11} is selected from OH, OC_{1-6}alkyl, $OC_{1-3}alkyl\text{-}O\text{-}C_{1-3}$alkyl, O-aryl, O-heterocyclyl, $O[C(O)CH(R_{14})NH]_sH$, $[sugar]_s$, SH, SC_{1-6}alkyl, $SC_{1-3}alkyl\text{-}O\text{-}C_{1-3}$alkyl, S-aryl, S-

heterocyclyl, $S[C(O)CH(R_{14})NH]_sH$, halo, $N(R_{15})_2$, $C(O)R_{16}$, CN, $C(R_{17})_3$, aryl and heterocyclyl;

R_{12} is selected from OH, SH, NH_2, halo, NO_2, $C(R_{17})_3$ and CN;

Each R_{13} is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl and $(A)_qR_{11}$;

R_{14} is the characterising group of an amino acid;

Each R_{15} is independently selected from hydrogen, C_{1-6}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aryl and heterocyclyl;

R_{16} is selected from C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

Each R_{17} is independently selected from hydrogen and halogen;

A is optionally substituted methylene wherein when n > 2, any two adjacent A groups are optionally interrupted by –O-, -S- or -$N(R_{15})$-;

where n is 0 or an integer selected from 1 to 20;
m is 0 or an integer selected from 1 to 3;
p is an integer selected from 1 to 20;
q is an integer selected from 1 to 10
s is an integer selected from 1 to 5;
t is an integer selected from 1 or 2; and

wherein each alkyl, alkenyl , alkynyl, aryl and heterocyclyl may be optionally substituted.

In another aspect, the invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine or biological activity is implicated comprising the administration of a treatment, prevention or diagnostic effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need

thereof.

In a further aspect, there is provided the use of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment, prevention or diagnosis of a disease or condition wherein MIF cytokine or biological activity is implicated.

In particular, the invention provides a method of treating, diagnosing or preventing autoimmune diseases, solid or haemopoitic tumours, or chronic or acute inflammatory diseases, including a disease or condition selected from the group comprising:

Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiostatis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg Alzheimers, multiple sclerosis), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation,

implantation, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing,

comprising the administration of a treatment, diagnosis or prevention effective amount of a compound of Formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

A further aspect of the invention provides for the use of a compound of Formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment of a disease or condition as above.

A further aspect of the invention provides a pharmaceutical composition comprising a compound of formula (I) and a pharmaceutically acceptable carrier, diluent or excipient.

In another aspect, the invention provides a method of treating or preventing a disease or condition wherein MIF cytokine or biological activity is implicated comprising:

administering to a mammal a compound of formula (I) and a second therapeutic agent.

In another aspect, the present invention provides a method of prophylaxis or treatment of a disease or condition for which treatment with a glucocorticoid is indicated, said method comprising:

administering to a mammal a glucocorticoid and a compound of formula (I).

In yet another aspect, the present invention provides a method of treating steroid-resistant diseases comprising:

administering to a mammal a glucocorticoid and a compound of formula (I).

In a further aspect, the present invention provides a method of enhancing the effect of a glucocorticoid in mammals comprising administering a compound of formula (I) simultaneously, separately or sequentially with said glucocorticoid.

In yet a further aspect, the present invention provides a pharmaceutical composition comprising a glucocorticoid and a compound of formula (I).

In a further aspect of the invention there is provided a use of a glucocorticoid in the manufacture of a medicament for administration with a compound of formula (I) for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

In yet a further aspect of the invention there is provided a use of a compound of formula (I) in the manufacture of a medicament for administration with a glucocorticoid for the treatment or prophylaxis of a disease or condition for which treatment of a glucocorticoid is indicated.

In yet a further aspect of the invention there is provided a use of a glucocorticoid and a compound of formula (I) in the manufacture of a medicament for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

The compounds of formula (I) or a pharmaceutically acceptable salt or prodrug thereof may also have an inhibitory effect on the tautomerase activity also associated with MIF. This may form a further aspect of the invention.

In preferred embodiments, the compounds of Formula (I) or a pharmaceutically acceptable salt or prodrug thereof are used to treat or prevent a disease or condition, particularly in a human subject.

Certain compounds of Formula (I) are novel, and these form a further aspect of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1: graphically depicts inhibition of MIF-induced proliferation of S112 human fibroblasts by 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1).

Figure 2: graphically depicts inhibition of IL-1 induced COX-2 expression by 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1).

Figure 3: graphically depicts inhibition of IL-1 induced COX-2 expression by 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1).

Figure 4: graphically depicts inhibition of IL-1 induced COX-2 expression by 2-(2-hydroxyethoxy)-2-(4'-hydroxyphenyl)-1,3-dioxolane (Compound 2).

Figure 5: graphically depicts inhibition of antigen-specific T-cell activation by 2-(2-hydroxyethoxy)-2-(4-hydroxyphenyl)-1,3-dioxolane (Compound 2).

Figure 6: graphically depicts the enhanced effect of the glucocorticoid dexamethasone in the presence of 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1).

Figure 7: graphically depicts the effects of 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1) on cell viability and apoptosis.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "alkyl" refers to monovalent straight, branched or, where appropriate, cyclic aliphatic radicals, having 1 to 3, 1 to 6, 1 to 10 or 1 to 20 carbon atoms, e.g. methyl, ethyl, n-propyl, iso-propyl, cyclopropyl, n-butyl, sec-butyl, t-butyl and cyclobutyl, n-pentyl, 1-methylbutyl, 2-methylbutyl, 3-methylbutyl, cyclopentyl, n-hexyl, 1- 2- 3- or 4- methylpentyl, 1- 2- or 3-ethylbutyl, 1 or 2- propylpropyl or cyclohexyl.

An alkyl group may be optionally substituted one or more times by halo (eg chloro, fluoro

or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, CO_2NH_2, $CO_2NH(C_{1-6}alkyl)$, $CO_2N_{(1-6)2}$, OH, alkoxy, acyl, acetyl, halomethyl, trifluoromethyl, benzyloxy, phenoxy, NH_2, $NH(C_{1-6}alkyl)$ or $NH(C_{1-6}alkyl)_2$. A preferred optional substituent is a polar substituent. Examples of alkoxy include methoxy, ethoxy, *n*-propoxy, *iso*-propoxy, cyclopropoxy, and butoxy (*n*-, *sec*- *t*- and cyclo) pentoxy and hexyloxy. The "alkyl" portion of an alkoxy group may be substituted as described above.

As used herein, the term "alkenyl" refers to straight, branched, or where appropriate, cyclic carbon containing radicals having one or more double bonds between carbon atoms. Examples of such radicals include vinyl, allyl, butenyl, or longer carbon chains such as those derived from palmitoleic, oleic, linoleic, linolenic or arachidonic acids. An alkenyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, CO_2NH_2, $CO_2NH(C_{1-6}alkyl)$, $CO_2N_{(1-6)2}$, OH, alkoxy, acyl, acetyl, halomethyl, trifluoromethyl, benzyloxy, phenoxy, NH_2, $NH(C_{1-6}alkyl)$ or $NH(C_{1-6}alkyl)_2$. A preferred optional substituent is a polar substituent.

As used herein, the term "alkynyl" refers to straight or branched carbon containing radicals having one or more triple bonds between carbon atoms. Examples of such radicals include propargyl, butynyl and hexynyl. An alkynyl group may be optionally substituted one or more times by halo (eg chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, CO_2NH_2, $CO_2NH(C_{1-6}alkyl)$, $CO_2N_{(1-6)2}$, OH, alkoxy, acyl, acetyl, halomethyl, trifluoromethyl, benzyloxy, phenoxy, NH_2, $NH(C_{1-6}alkyl)$ or $NH(C_{1-6}alkyl)_2$. A preferred optional substituent is a polar substituent.

Examples of suitable NH(alkyl) and $N(alkyl)_2$ include methylamino, ethylamino, isopropylamino, dimethylamino, n-propylamino, diethylamino and di-isopropylamino.

The term "halogen" (or "halo") refers to fluorine (fluoro), chlorine (chloro), bromine (bromo) or iodine (iodo).

The term "sugar" refers to a pyranosyl or furanosyl moiety such as those derived from glucose, galactose, mannose, allose, altrose, gluose, idose, talose, ribose, arabinose or xylose. Derivatives of such sugars include deoxy or amino pyranosyl or furanosyl sugar derivatives. Each sugar moiety is incorporated into the compound of formula (I) through a

hydroxy group of the sugar moiety.

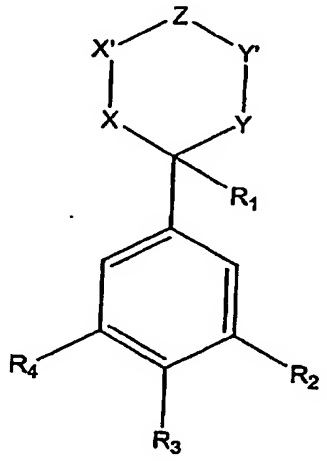
As used herein, "the characterising group of an amino acid" refers to the substituent at C_2 of a natural or unnatural amino acid and which defines the amino acid. For example, methyl is the characterising group of alanine, phenylmethyl is the characterising group of phenylalanine, hydroxymethyl is the characterising group of serine, hydroxyethyl is the characterising group of homoserine and n-propyl is the characterising group of norvaline.

An aryl group, as used herein, refers to C_6-C_{10} aryl groups such as phenyl or naphthalene. Aryl groups may be optionally substituted one or more times by halo (eg, chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, CO_2NH_2, $CO_2NH(C_{1-6}alkyl)$, $CO_2N(_{1-6})_2$, OH, alkoxy, acyl, acetyl, halomethyl, trifluoromethyl, benzyloxy, phenoxy, NH_2, $NH(C_{1-6}alkyl)$ or $NH(C_{1-6}alkyl)_2$.

As used herein, the term "heterocyclyl" refers to a cyclic, aliphatic or aromatic radical containing at least one heteroatom independently selected from O, N or S. Examples of suitable heterocyclyl groups include furyl, dioxolanyl, dioxanyl, dithianyl, dithiolanyl, pyridinyl, pyrimidinyl, pyrazolyl, piperidinyl, pyrrolyl, thyaphenyl, oxazolyl, imidazolyl, thiazolyl, isoxazolyl, isothiazolyl, quinolyl, isoquinolyl, indolyl, benzofuranyl, benzothiophenyl, triazolyl, tetrazolyl, oxadiazolyl and purinyl. Heterocyclyl groups may be optionally substituted one or more times by halo (eg, chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, CO_2NH_2, $CO_2NH(C_{1-6}alkyl)$, $CO_2N(_{1-6})_2$, OH, alkoxy, acyl, acetyl, halomethyl, trifluoromethyl, benzyloxy, phenoxy, NH_2, $NH(C_{1-6}alkyl)$ or $NH(C_{1-6}alkyl)_2$.

Each A is an unsubstituted methylene group ($-CH_2-$) or an optionally substituted methylene group where one or two of the hydrogen atoms of the methylene group may be replaced by a substituent, such as halo (eg. chloro, fluoro or bromo), CN, NO_2, CO_2H, CO_2C_{1-6}alkyl, CO_2NH_2, $CO_2NH(C_{1-6}alkyl)$, $CO_2N(_{1-6})_2$, OH, alkoxy, acyl, acetyl, halomethyl, trifluoromethyl, benzyloxy, phenoxy, NH_2, $NH(C_{1-6}alkyl)$ or $NH(C_{1-6}alkyl)_2$. $(A)_n$ may therefore form an optionally substituted methylene group, when n is 1, or an optionally substituted alkylene group when n is greater than 1. Alternatively, when two or more A groups appear in adjacent positions, they are optionally interrupted by –O-, -S- or $-N(R_{15})-$. $(A)_n$ may therefore form, for example, an optionally substituted ether or polyether.

In a first aspect, the present invention provides a method of inhibiting cytokine or biological activity of MIF comprising contacting MIF with a cytokine or biological activity inhibiting effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof



(I)

wherein X and X' are independently selected from $-C(R_5)_2-$, –O-, -S-, $-N(R_5)-$, or taken together form $-C(R_5)=C(R_5)-$, $-C(R_5)=N-$, $-N=C(R_5)$, $-N(R_5)-N(R_5)-$ or –N=N-;

Y and Y' are independently selected from $-C(R_5)_2-$, –O-, -S-, $-N(R_5)-$, or taken together form $-C(R_5)=C(R_5)-$, $-C(R_5)=N-$, $-N=C(R_5)$, $-N(R_5)-N(R_5)-$ or –N=N-;

Z is $-C(R_5)_2-$, -O-, -S- or $-N(R_5)-$, or forms a covalent single or double bond between X' and Y', or Z together with X' or Y' forms $-C(R_5)=C(R_5)-$, $-C(R_5)=N-$, $-N=C(R_5)$, $-N(R_5)-N(R_5)-$ or –N=N-;

wherein when Z is –O-, -S- or $-N(R_5)-$, X' and Y' are $-C(R_5)_2$;

when X is –O-, -S- or $-N(R_5)-$, X' is $-C(R_5)_2$;

when Y is –O-, -S- or $-N(R_5)-$, Y' is $-C(R_5)_2$; or

X or Y together with the carbon atom bearing the phenyl group form a double bond;

R_1 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_nC(O)R_6$, $(A)_nC(S)R_6$, $(A)_nS(O)R_6$, $(A)_nS(O)_2R_6$, $(A)_nOR_7$, $(A)_nSR_7$, $(A)_nN(R_8)$, $(A)_nC(=NR_9)R_{10}$ and $(A)_nR_{11}$, or when X or Y together with the carbon atom bearing the phenyl group form a double bond, R_1 is absent;

R_2 and R_4 are independently selected from hydrogen, C_{1-3}alkyl and $(A)_mR_{12}$;

R_3 is selected from C_{1-3}alkyl, $(A)_mR_{12}$, $(A)_m$aryl and $(A)_m$heterocyclyl;

R_5 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_nC(O)R_6$, $(A)_nC(S)R_6$, $(A)_nS(O)R_6$, $(A)_nS(O)_2R_6$, $(A)_nOR_7$, $(A)_nSR_7$, $(A)_pN(R_8)$, $(A)_nC(=NR_9)R_{10}$ and $(A)_nR_{11}$;

R_6 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, OH, OC_{1-10}alkyl, OC_{2-10}alkenyl, OC_{2-10}alkynyl, $O(A)_qR_{11}$, SH, SC_{1-10}alkyl, SC_{2-10}alkenyl, SC_{2-10}alkynyl, $S(A)_qR_{11}$, $N(R_{13})_2$, $[NH\text{-}CH(R_{14})C(O)]_s\text{-}OH$, $[NH\text{-}CH(R_{14})C(O)]_s\text{-}OC_{1-3}$alkyl, $[sugar]_s$ and $(A)_qR_{11}$;

R_7 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_qR_{11}$, C(O)H, $C(O)C_{1-10}$alkyl, $C(O)C_{2-10}$alkenyl, $C(O)C_{2-10}$alkynyl, C(O)-aryl, $C(O)(A)_qR_{11}$, $C(O)_2H$, $C(O)_2C_{1-10}$alkyl, $C(O)_2C_{2-10}$alkenyl, $C(O)_2C_{2-10}$alkynyl, $C(O)_2$-aryl, $C(O)_2(A)_qR_{11}$, C(S)H, $C(S)C_{1-10}$alkyl, $C(S)C_{2-10}$alkenyl, $C(S)C_{2-10}$alkynyl, C(S)-aryl, $C(S)(A)_qR_{11}$, C(S)OH, $C(S)OC_{1-10}$alkyl, $C(S)OC_{2-10}$alkenyl, $C(S)OC_{2-10}$alkynyl, C(S)O-aryl, $C(S)O(A)_qR_{11}$, $S(O)_tH$, $S(O)_tC_{1-10}$alkyl, $S(O)_tC_{2-10}$alkenyl, $S(O)_tC_{2-10}$alkynyl, $S(O)_t$-aryl, $S(O)_t(A)_qR_{11}$, $[C(O)CH(R_{14})NH]_s\text{-}H$, $[C(O)CH(R_{14})NH]_s\text{-}C_{1-10}$alkyl, $[C(O)CH(R_{14})NH]_s\text{-}C_{2-10}$alkenyl, $[C(O)CH(R_{14})NH]_s\text{-}C_{2-10}$alkynyl, $[C(O)CH(R_{14})NH]_s$-aryl, $[C(O)CH(R_{14})NH]_s\text{-}(A)_qR_{11}$ and $[sugar]_s$;

Each R_8 is independently selected from R_7 and $NHC(=NR_{15})NH_2$;

R_9 is selected from hydrogen and C_{1-6}alkyl;

R_{10} is selected from C_{1-6}alkyl, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, OH, OC_{1-3}alkyl, SH and SC_{1-3}alkyl;

R_{11} is selected from OH, OC_{1-6}alkyl, OC_{1-3}alkyl-O-C_{1-3}alkyl, O-aryl, O-heterocyclyl, $O[C(O)CH(R_{14})NH]_sH$, $[sugar]_s$, SH, SC_{1-6}alkyl, SC_{1-3}alkyl-O-C_{1-3}alkyl, S-aryl, S-heterocyclyl, $S[C(O)CH(R_{14})NH]_sH$, halo, $N(R_{15})_2$, $C(O)R_{16}$, CN, $C(R_{17})_3$, aryl and heterocyclyl;

R_{12} is selected from OH, SH, NH_2, halo, NO_2, $C(R_{17})_3$ and CN;

Each R_{13} is independently selected from hydrogen, C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl and $(A)_qR_{11}$;

R_{14} is the characterising group of an amino acid;

Each R_{15} is independently selected from hydrogen, C_{1-6}alkyl, C_{1-3}alkoxyC_{1-3}alkyl, aryl and heterocyclyl;

R_{16} is selected from C_{1-3}alkyl, OH, C_{1-3}alkoxy, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

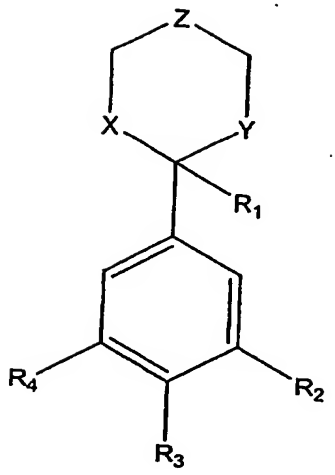
Each R_{17} is independently selected from hydrogen and halogen;

A is optionally substituted methylene wherein when $n > 2$, any two adjacent A groups are optionally interrupted by –O-, -S- or -$N(R_{15})$-;

where n is 0 or an integer selected from 1 to 20;
m is 0 or an integer selected from 1 to 3;
p is an integer selected from 1 to 20;
q is an integer selected from 1 to 10
s is an integer selected from 1 to 5;
t is an integer selected from 1 or 2; and

wherein each alkyl, alkenyl , alkynyl, aryl and heterocyclyl may be optionally substituted.

In another aspect, the compound of the invention is a compound of formula (II), or a pharmaceutically acceptable salt or prodrug thereof



(II)

wherein X and Y are independently selected from –O-, -S-, -N(R_5)- and –C(R_5)$_2$-;

Z is –C(R_5)$_2$- or is a covalent bond between adjacent methylene groups;

R_1 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_nC(O)R_6$, $(A)_nC(S)R_6$, $(A)_nS(O)R_6$, $(A)_nS(O)_2R_6$, $(A)_nOR_7$, $(A)_nSR_7$, $(A)_nN(R_8)$, $(A)_nC(=NR_9)R_{10}$ and $(A)_nR_{11}$;

R_2 and R_4 are independently selected from hydrogen, C_{1-3}alkyl and $(A)_mR_{12}$;

R_3 is selected from C_{1-3}alkyl, $(A)_mR_{12}$, $(A)_m$aryl and $(A)_m$heterocyclyl;

R_5 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_nC(O)R_6$, $(A)_nC(S)R_6$, $(A)_nS(O)R_6$, $(A)_nS(O)_2R_6$, $(A)_nOR_7$, $(A)_nSR_7$, $(A)_pN(R_8)$, $(A)_nC(=NR_9)R_{10}$ and $(A)_nR_{11}$;

R_6 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, OH, OC_{1-10}alkyl, OC_{2-10}alkenyl, OC_{2-10}alkynyl, $O(A)_qR_{11}$, SH, SC_{1-10}alkyl, SC_{2-10}alkenyl, SC_{2-10}alkynyl, $S(A)_qR_{11}$, $N(R_{13})_2$, $[NH\text{-}CH(R_{14})C(O)]_s$-OH, $[NH\text{-}CH(R_{14})C(O)]_s$-$OC_{1-3}$alkyl, $[sugar]_s$ and $(A)_qR_{11}$;

R_7 is selected from hydrogen, C_{1-20}alkyl, C_{2-20}alkenyl, C_{2-20}alkynyl, $(A)_qR_{11}$, C(O)H, $C(O)C_{1-10}$alkyl, $C(O)C_{2-10}$alkenyl, $C(O)C_{2-10}$alkynyl, C(O)-aryl, $C(O)(A)_qR_{11}$, $C(O)_2H$, $C(O)_2C_{1-10}$alkyl, $C(O)_2C_{2-10}$alkenyl, $C(O)_2C_{2-10}$alkynyl, $C(O)_2$-aryl, $C(O)_2(A)_qR_{11}$, C(S)H, $C(S)C_{1-10}$alkyl, $C(S)C_{2-10}$alkenyl, $C(S)C_{2-10}$alkynyl, C(S)-aryl, $C(S)(A)_qR_{11}$, C(S)OH,

$C(S)OC_{1-10}alkyl$, $C(S)OC_{2-10}alkenyl$, $C(S)OC_{2-10}alkynyl$, C(S)O-aryl, $C(S)O(A)_qR_{11}$, $S(O)_tH$, $S(O)_tC_{1-10}alkyl$, $S(O)_tC_{2-10}alkenyl$, $S(O)_tC_{2-10}alkynyl$, $S(O)_t$-aryl, $S(O)_t(A)_qR_{11}$, $[C(O)CH(R_{14})NH]_s$-H, $[C(O)CH(R_{14})NH]_s$-$C_{1-10}alkyl$, $[C(O)CH(R_{14})NH]_s$-$C_{2-10}alkenyl$, $[C(O)CH(R_{14})NH]_s$-$C_{2-10}alkynyl$, $[C(O)CH(R_{14})NH]_s$-aryl, $[C(O)CH(R_{14})NH]_s$-$(A)_qR_{11}$ and $[sugar]_s$;

Each R_8 is independently selected from R_7 and $NHC(=NR_{15})NH_2$;

R_9 is selected from hydrogen and $C_{1-6}alkyl$;

R_{10} is selected from $C_{1-6}alkyl$, NH_2, $NH(C_{1-3}alkyl)$, $N(C_{1-3}alkyl)_2$, OH, $OC_{1-3}alkyl$, SH and $SC_{1-3}alkyl$;

R_{11} is selected from OH, $OC_{1-6}alkyl$, $OC_{1-3}alkyl$-O-$C_{1-3}alkyl$, O-aryl, O-heterocyclyl, $O[C(O)CH(R_{14})NH]_sH$, $[sugar]_s$, SH, $SC_{1-6}alkyl$, $SC_{1-3}alkyl$-O-$C_{1-3}alkyl$, S-aryl, S-heterocyclyl, $S[C(O)CH(R_{14})NH]_sH$, halo, $N(R_{15})_2$, $C(O)R_{16}$, CN, $C(R_{17})_3$, aryl and heterocyclyl;

R_{12} is selected from OH, SH, NH_2, halo, NO_2, $C(R_{17})_3$ and CN;

Each R_{13} is independently selected from hydrogen, $C_{1-6}alkyl$, $C_{2-6}alkenyl$, $C_{2-6}alkynyl$ and $(A)_qR_{11}$;

R_{14} is the characterising group of an amino acid;

Each R_{15} is independently selected from hydrogen, $C_{1-6}alkyl$, $C_{1-3}alkoxyC_{1-3}alkyl$, aryl and heterocyclyl;

R_{16} is selected from $C_{1-3}alkyl$, OH, $C_{1-3}alkoxy$, aryl, aryloxy, heterocyclyl and heterocyclyloxy;

Each R_{17} is independently selected from hydrogen and halogen;

A is optionally substituted methylene wherein when n > 2, any two adjacent A groups are optionally interrupted by –O-, -S- or -N(R_{15})-;

where n is 0 or an integer selected from 1 to 20;
m is 0 or an integer selected from 1 to 3;
p is an integer selected from 1 to 20;
q is an integer selected from 1 to 10
s is an integer selected from 1 to 5;
t is an integer selected from 1 or 2; and

wherein each alkyl, alkenyl , alkynyl, aryl and heterocyclyl may be optionally substituted.

In a preferred embodiment one or more of the following definitions apply:

X is -O-, -S-, -NH- or -CH_2-;

Y is -O-, -S- or –NR_5-;

Z forms a covalent bond between adjacent methylene groups;

R_1 is selected from C_{1-20}alkyl, C_{1-20}alkenyl, O-$(A)_q$O-C_{1-6}alkyl, O-$(A)_q$-heterocyclyl, O-$(A)_q$-sugar, O-$(A)_q$O[C(O)CH(R_{14})NH]$_s$-H, $(A)_n$OH, $(A)_n$OC_{1-20}alkyl, $(A)_n$OC_{1-20}alkenyl, $(A)_n$OC(O)C_{1-20}alkyl, $(A)_n$OC(O)C_{1-20}alkenyl, $(A)_n$OC(O)aryl, $(A)_n$O[C(O)CH(R_{14})NH]$_s$-H, $(A)_n$O[sugar]$_s$, $(A)_n$NHC_{1-20}alkyl, $(A)_n$N(C_{1-20}alkyl)$_2$, $(A)_n$NHC_{1-20}alkenyl, $(A)_n$N(C_{1-20}alkenyl)$_2$, $(A)_n$NHC(O)C_{1-20}alkyl, $(A)_n$NHC(O)C_{1-20}alkenyl, $(A)_n$NHC(O)aryl, $(A)_n$NH[C(O)CH(R_{14})NH]$_s$-H, $(A)_n$NH-[sugar]$_s$, $(A)_n$$SO_3$H, $(A)_n$$SO_3$$C_{1-20}$alkyl, $(A)_n$$SO_3$$C_{1-20}$alkenyl, $(A)_n$C(O)C_{1-20}alkyl, $(A)_n$C(O)C_{1-20}alkenyl, $(A)_n$$CO_2$H, $(A)_n$$CO_2$$C_{1-20}$alkyl, $(A)_n$$CO_2$$C_{1-20}$alkenyl, $(A)_n$C(O)NHC_{1-20}alkyl, $(A)_n$C(O)N(C_{1-20}alkyl)$_2$, $(A)_n$C(O)NHC_{1-20}alkenyl, $(A)_n$C(O)N(C_{1-20}alkenyl)$_2$, $(A)_n$C(O)[NHCH(R_{14})C(O)]$_s$-OH, $(A)_n$C(O)[sugar]$_s$; wherein A is methylene optionally substituted one or two times with a group that is independently selected from C_{1-6}alkyl, C_{2-6}alkenyl, C_{2-6}alkynyl, halogen, OH, OC_{1-6}alkyl, CO_2H, $CO_2$$C_{1-3}$alkyl, NH_2, NHC_{1-3}alkyl, -N(C_{1-3}alkyl)$_2$, CN, NO_2, aryl or heterocyclyl; R_{14} is the characterising group of an amino acid, n is 0 or an integer from 1 to 20 and s is an integer

from 1 to 5;

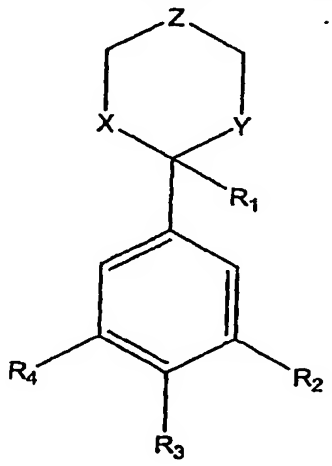
R_2 is hydrogen, C_{1-3} alkyl, OH, SH, NH_2, $-NO_2$, CF_3, halo or -CN;

R_3 is hydrogen, C_1-C_3alkyl, $-(CH_2)_mNH_2$, $-(CH_2)_m$-OH, $-(CH_2)_m$-CF_3, $-(CH_2)_m$-SH or a 5 or 6 membered heterocyclic group, wherein m is 0 or an integer from 1 to 3;

R_4 is hydrogen, C_{1-3}alkyl, OH, SH, NH_2, NO_2, CF_3, halo or CN;

A is unsubstituted methylene or mono-substituted methylene.

In certain preferred forms of the invention, the compounds of Formula (II) include:



(II)

wherein

X is -O-, -S-, -NH-;

Y is -O-, -S- or $-N(R_5)-$;

Z forms a covalent bond between adjacent methylene groups;

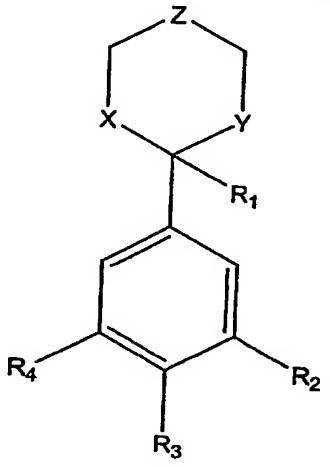
R_1 is C_1-C_{20}alkyl, C_2-C_{20}alkenyl, C_2-C_{20}alkynyl, $(A)_nC(O)R_6$, $-(A)_nC(S)R_6$, $-(A)_nS(O)R_6$, $-(A)_nS(O)_2R_6$, $-(A)_nOR_7$, $-(A)_nSR_7$, $-(A)_nN(R_8)_2$, $(A)_nC(=NR_9)R_{10}$ or $(A)_nR_{11}$ where n, R_6, R_7, R_8, R_9, R_{10} and R_{11} are defined above;

R_2 is hydrogen, methyl, OH, SH, NH_2, NO_2, CF_3, halo or CN;

R_3 is C_{1-3}alkyl, $-(CH_2)_mNH_2$, $-(CH_2)_m$-OH, $-(CH_2)_mSH$ or heterocyclyl where m is defined above;

R_4 is hydrogen, methyl, OH, SH, NH_2, NO_2, CF_3, CF_3, halo or CN.

More preferably the compounds of formula (II) comprise



(II)

wherein

X is -O- or NH;

Y is -O- or $-N(R_{18})-$ where R_{18} is selected from hydrogen, C_{1-20}alkyl, C_{1-20}alkenyl, C_{1-20}alkenyl, C_{1-20}alkynyl and $(CH_2)_nR_{11}$ where R_{11} and n are defined above;

Z forms a covalent bond between adjacent methylene groups;

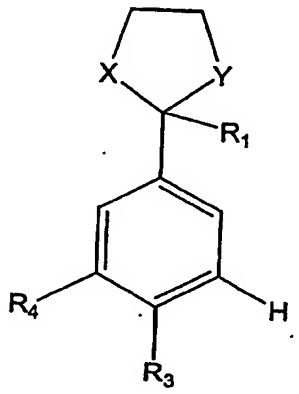
R_1 is as defined for R_1 above;

R_2 is hydrogen, halomethyl, OH, SH, NH_2, NO_2 or CN;

R_3 is hydrogen, C_{1-3}alkyl, $(CH_2)_mNH_2$, $(CH_2)_mOH$ or $(CH_2)_mCF_3$ or heterocyclyl where m is defined above;

R_4 is hydrogen, methyl, OH, SH, NH_2, NO_2 or CN.

More preferably, the compounds of Formula (I) are heterocyclic compounds having the formula (III)



(III)

wherein

X is -O- or -NH-;

Y is -O- or -N(R_{18})- where R_{18} is defined above;

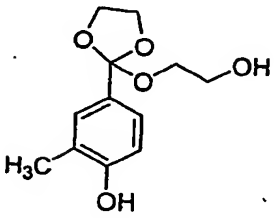
R_1 is as defined for R_1 above;

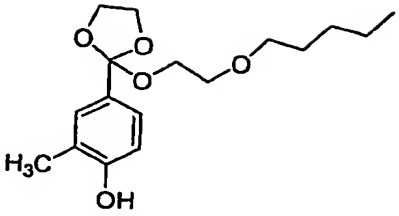
R_3 is hydrogen, NH_2, OH;

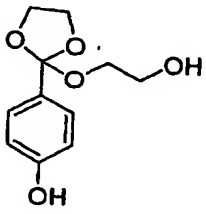
R_4 is hydrogen, methyl, or OH.

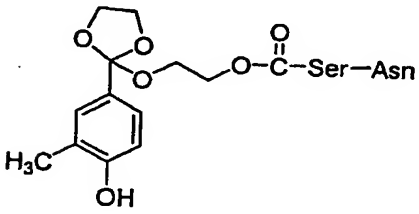
In a preferred embodiment R_1 is selected from $(A)_nOR_7$ where n is 0 and A and R_7 are defined above.

Examples of suitable compounds may include

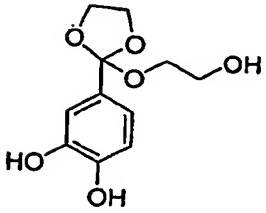


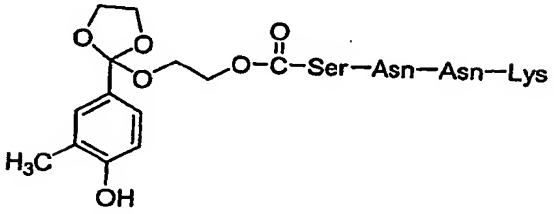




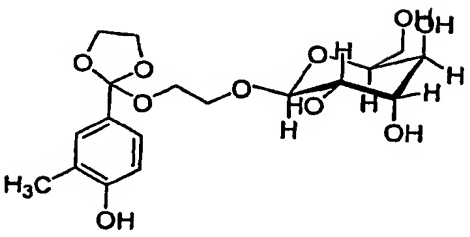


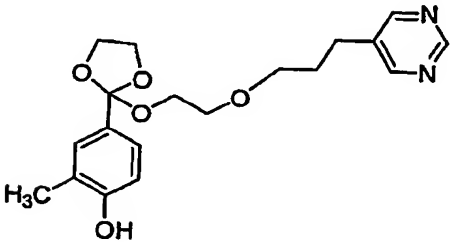
Ser—Asn

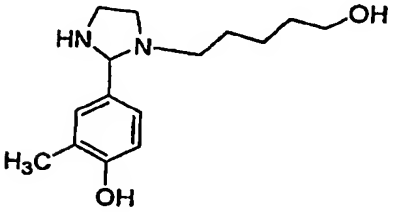


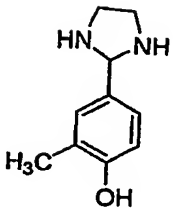


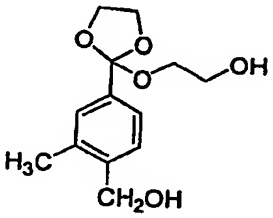
Ser—Asn—Asn—Lys







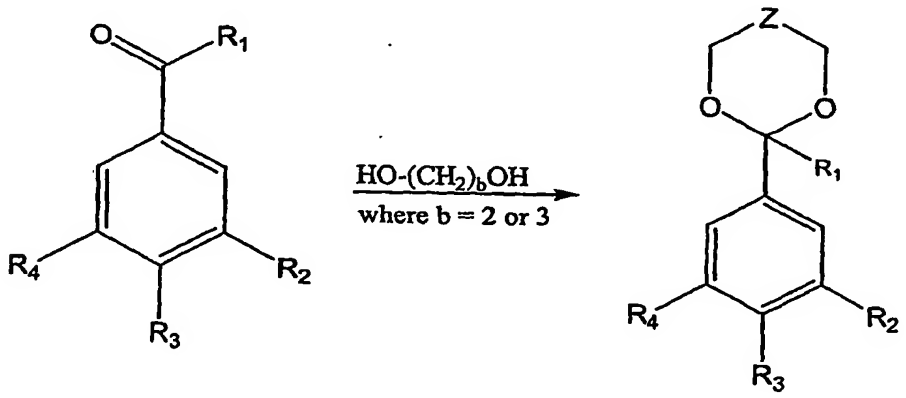




CH2OH

Compounds of Formula (I) may be prepared using the methods depicted or described herein or known in the art. It will be understood that minor modifications to methods described herein or known in the art may be required to synthesize particular compounds of Formula (I). General synthetic procedures applicable to the synthesis of compounds may be found in standard references such as *Comprehensive Organic Transformations*, R. C. Larock, 1989, VCH Publishers and *Advanced Organic Chemistry*, J. March, 4th Edition (1992), Wiley InterScience, and references therein. It will also be recognised that certain reactive groups may require protection and deprotection during the synthetic process. Suitable protecting and deprotecting methods for reactive functional groups are known in the art for example in *Protective Groups in Organic Synthesis*, T. W. Green & P. Wutz, John Wiley & Son, 3rd Edition, 1999.

Thus for certain embodiments of the invention, compounds of formula (I), where X and Y are -O-, X' and Y' are $-CH_2-$, Z is $-CH_2-$ or forms a bond between X' and Y' and R_1 is alkyl, alkenyl, alkynyl or an optionally substituted alkylene with terminal functionality, eg $(A)_nOMe$ where n is between 1 and 20, may be prepared by the general method shown in Scheme 1.



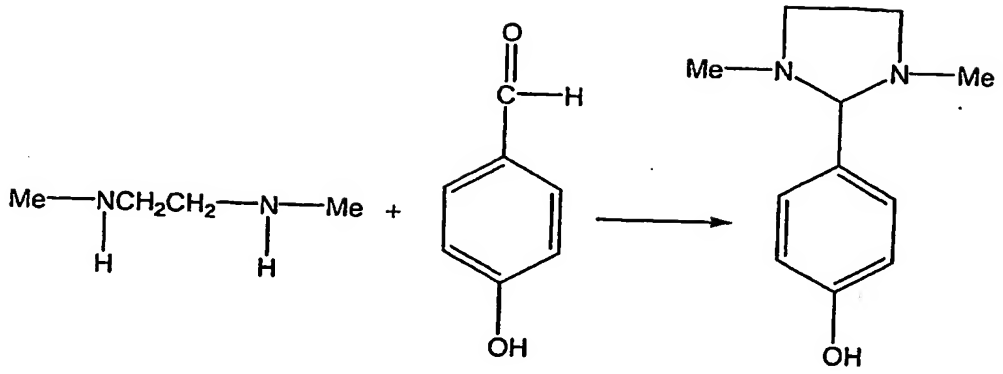
Scheme 1

Suitable starting materials may be commercially available or made by methods known in the art. Suitable conditions for this reaction include refluxing the starting material and the dihydroxy compound in benzene in the presence of acid, eg. tosylate. Other conditions for performing this reaction to provide selectivity in the presence of other carbonyl groups or to provide conditions suitable for use in the presence of other functional groups are provided in *Protective Groups in Organic Synthesis*, T.W. Green & P. Wutz, John Wiley & Son; 3rd Edition, 1999, pages 312-329. Functionality may be introduced into the dioxolane group by using a substituted dihydroxy compound.

1,3-dithiane or 1,3-dithiolane derivatives, where X and Y are -S-, X' and Y' are $-CH_2-$, Z is $-CH_2-$ or forms a covalent bond between X' and Y' and R_1 is alkyl, alkenyl, alkynyl or an optionally substituted alkylene with terminal functionality, eg.: $(A)_nOMe$ where n is 1 to 20, may be prepared in a similar manner as the 1,3-dioxolane derivatives in Scheme 1. Suitable conditions for this reaction include mixing the starting material and $HS-(CH_2)_b-SH$, where b is 2 or 3, in the presence of BF_3-Et_2O in dichloromethane at room temperature. Other conditions for performing this reaction are provided in *Protective Groups in Organic Synthesis*, T.W. Green & P. Wutz, John Wiley & Son; 3rd Edition, 1999, pages 333-336.

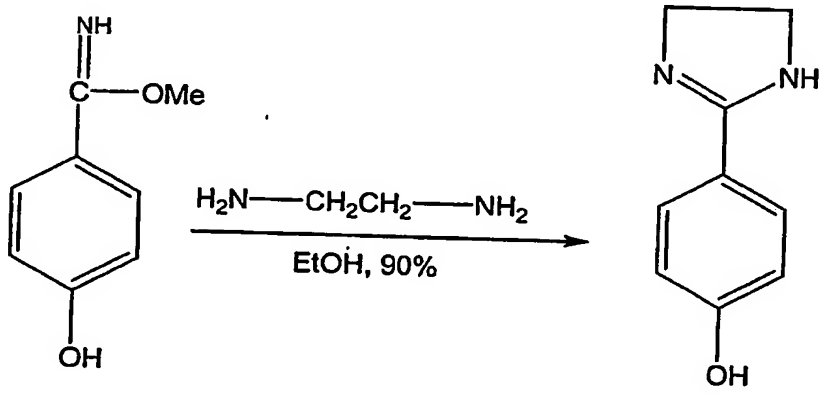
1,3-oxathiolanes, where one of X and Y is -O- and the other is -S-, X' and Y' are $-CH_2-$, Z is $-CH_2-$ or forms a covalent bond between X' and Y' and R_1 is alkyl, alkenyl, alkynyl or an optionally substituted alkylene with terminal functionality, eg.: $(A)_nOMe$ where n is 1 to 20, may be prepared in a similar manner as the 1,3-dioxolane derivatives in Scheme 1. Suitable conditions include mixing the starting material with $HS-(CH_2)_b-OH$ where b is 2 or 3, in dioxane, in the presence of $ZnCl_2$ and AcONa at room temperature. Conditions for performing this reaction are given in *Protective Groups in Organic Synthesis*, T.W. Green & P. Wutz, John Wiley & Son; 3rd Edition, 1999, at page 346.

Compounds where X and Y are $-N(R_5)-$, X' and Y' are $-CH_2-$, Z is $-CH_2-$ or forms a covalent bond between X' and Y' and R_1 is alkyl, alkenyl, alkynyl or an optionally substituted alkylene with terminal functionality, eg.: $(A)_nOMe$ where n is 1 to 20, may be prepared as shown in Scheme 2 (12):



Scheme 2

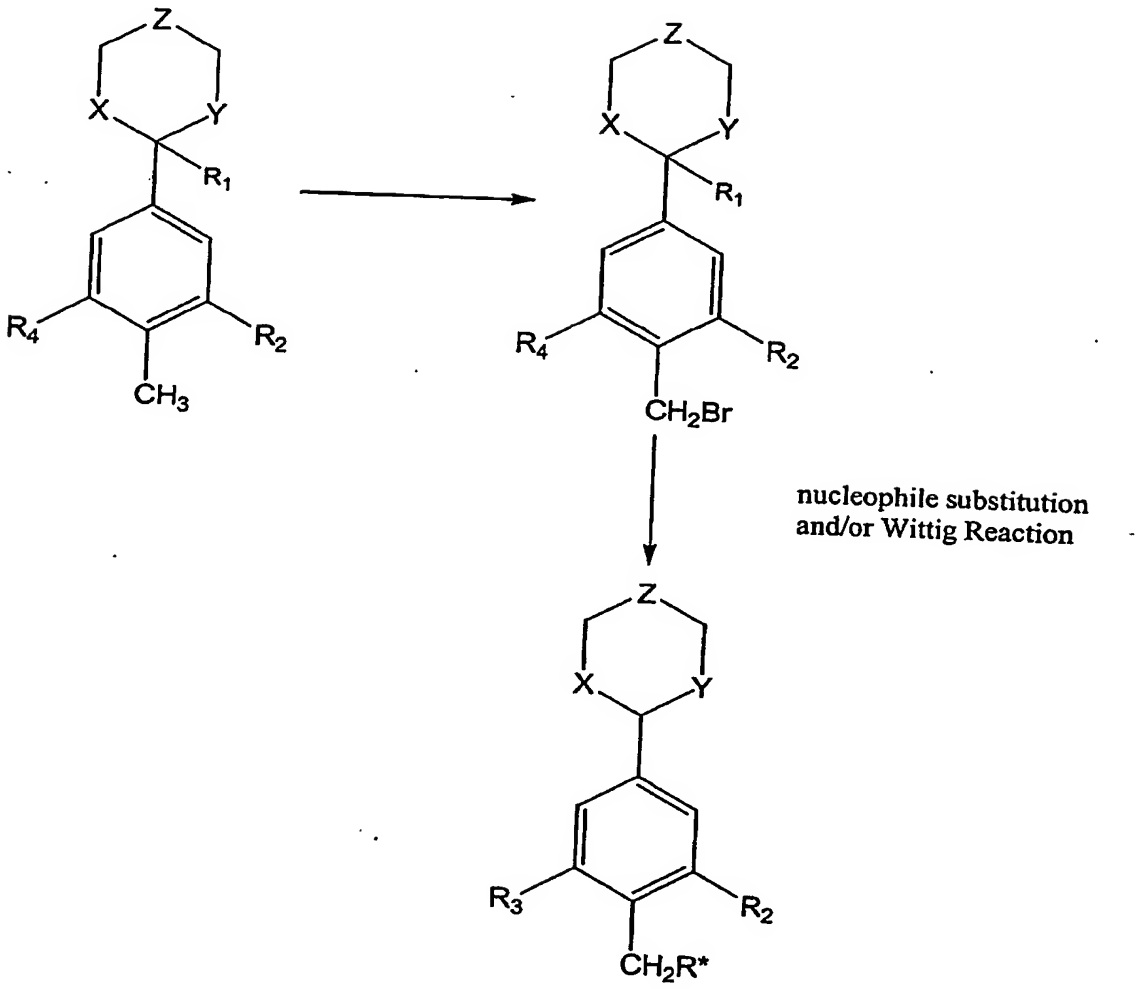
Compounds where X is -N(R_5)-, X' and Y' are -CH_2-, Z is -CH_2- or forms a covalent bond between X' and Y', Y together with the carbon atom to which the phenyl group is attached is a double bond and R_1 is absent may be prepared as shown in Scheme 3 (13).



Scheme 3

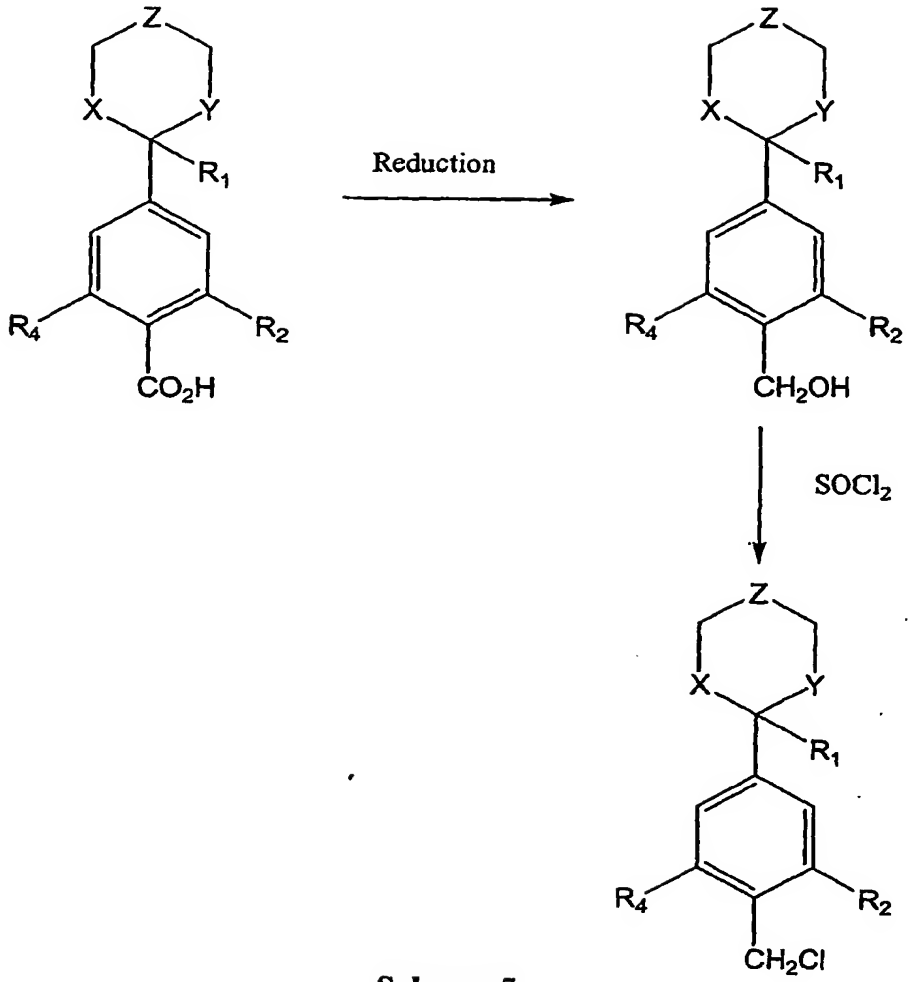
When R_1 includes a -CO_2H or -C(S)OH group, the compounds may be further derivatised to provide ketones, thioketones, esters, thioesters, amides and thioamides by standard alkylating, esterifying or amide forming methodology. When R_1 includes a hydroxy, thiol or amino group, these groups may be further derivatised to provide esters, thioesters, amides, ethers, thioethers and N-alkyl groups using standard acylating or alkylating methodology. Conversion of an amide to C=NH(NH_2) can be achieved by aminolysis eg NH_3/dry methanol.

In other embodiments, compounds of Formula (I), where R_1, R_2, R_3 or R_4 is a substituted methyl group, can be prepared by conversion of the methyl substituent into a halomethyl substituent (eg by treatment with a N-halosuccinimide such as NBS) followed by nucleophilic substitution by an appropriate nucleophile and/or insertion of additional methylene groups by, for example, Wittig reaction (see Scheme 4 where R^* can be, for example, $(CH_2)_xOH$, $(CH_2)_xSH$, $(CH_2)_xNH_2$, $(CH_2)_x$heterocyclyl, $(CH_2)_x$aryl, $(CH_2)_xNO_2$ where x is 0, 1 or 2. Similar rections could be performed if R_1 is CH_2Br to provide substituents such as $(CH_2)_nC(O)C_{1-20}$alkyl, $(CH_2)_nOC(O)C_{1-10}$alkyl, $(CH_2)_nOC_{1-20}$alkyl, $(CH_2)_n$Ophenyl, $(CH_2)_n$Obenzyl, $(CH_2)_nNHC_{1-20}$alkyl, $(CH_2)_nN(C_{1-20}alkyl)_2$, $(CH_2)_n$NHphenyl, $(CH_2)_n$NHbenzyl, $(CH_2)_nSC_{1-20}$alkyl, $(CH_2)_nSC(O)C_{1-10}$alkyl, $(CH_2)_n$Sphenyl, $(CH_2)_n$Sbenzyl, $(CH_2)_n$NHsugar, $(CH_2)_n$Ssugar, $(CH_2)_n$Osugar, $(CH_2)_nNHC(O)C_{1-10}$alkyl, $(CH_2)_n$NHC(O)phenyl, $(CH_2)_n$NHC(O)benzyl, $(CH_2)_nNHCO_2C_{1-6}$alkyl, $(CH_2)_nNHCO_2$phenyl, or $(CH_2)_nNHCO_2$benzyl , where n is 0 or 1 to 20).



Scheme 4

In other embodiments, compounds where R_1, R_2, R_3 or R_4 are CH_2halo can be prepared by reaction of a suitable carboxylic acid derivative with a reducing agent such as $LiAlH_4$, followed by halogenation, eg treatment with thionyl chloride (Scheme 5).

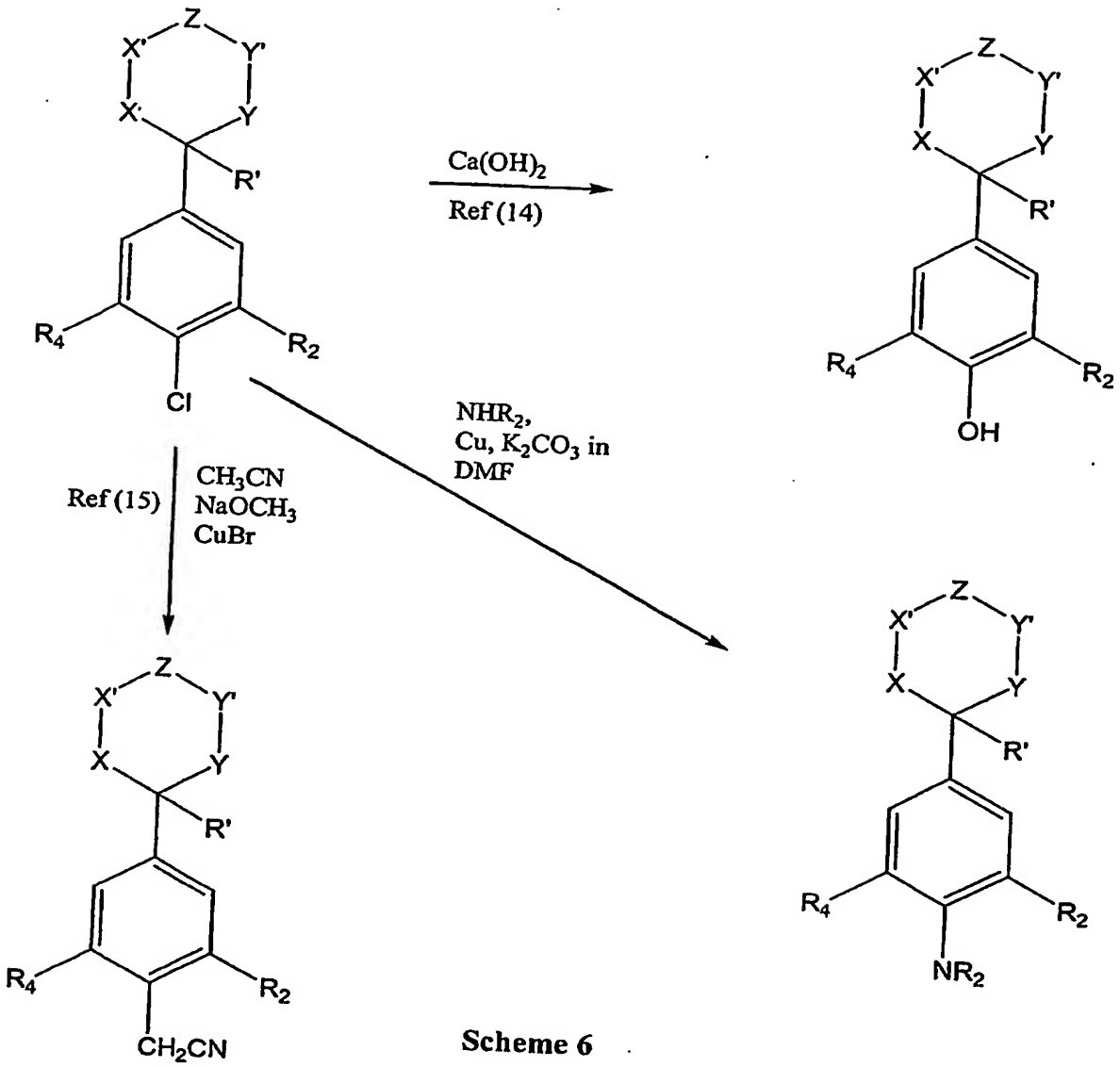


Scheme 5

Coupling of compounds wherein R_1, R_2, R_3 or R_4 is CH_2halo with an alkylhalide or halo$(CH_2)_a$heterocyclyl in the presence of CuLi affords the corresponding compounds where the R_1, R_2, R_3 or R_4 substituent is alkyl or where R_1 or R_3 are $(CH_2)_a$heterocyclyl where a is 1-20 in relation to R_1 or 1 to 3 in relation to R_3.

Reaction of CH_2halo with NH_2-NH-C(=NH)-NH_2 in the presence of base affords access to compounds wherein R_1 is CH_2-NH-NH-C(=NH)-NH_2. Alternatively, reaction of the CH_2halo group with halo$(CH_2)_p$NH-NH-C(=NH)-NH_2 (where p is 1 or 2), affords the group $(CH_2)_p$NH-NH-C(=NH)-NH2 where p is 2 or 3.

Compounds where R_3 is -OH, -NR or -CH_2CN can be prepared from the compound where R_3 is Cl as shown in Scheme 6



Scheme 6

The term "salt, or prodrug" includes any pharmaceutically acceptable salt, ester, solvate, hydrate or any other compound which, upon administration to the recipient is capable of providing (directly or indirectly) a compound of Formula (I) as described herein. The term "pro-drug" is used in its broadest sense and encompasses those derivatives that are converted *in vivo* to the compounds of the invention. Such derivatives would readily occur to those skilled in the art, and include, for example, compounds where a free hydroxy group is converted into an ester, such as an acetate, or where a free amino group is converted into an amide. Procedures for acylating hydroxy or amino groups of the compounds of the invention are well known in the art and may include treatment of the

compound with an appropriate carboxylic acid, anhydride or acylchloride in the presence of a suitable catalyst or base.

Suitable pharmaceutically acceptable salts include, but are not limited to, salts of pharmaceutically acceptable inorganic acids such as hydrochloric, sulphuric, phosphoric, nitric, carbonic, boric, sulfamic, and hydrobromic acids, or salts of pharmaceutically acceptable organic acids such as acetic, propionic, butyric, tartaric, maleic, hydroxymaleic, fumaric, maleic, citric, lactic, mucic, gluconic, benzoic, succinic, oxalic, phenylacetic, methanesulphonic, toluenesulphonic, benezenesulphonic, salicyclic sulphanilic, aspartic, glutamic, edetic, stearic, palmitic, oleic, lauric, pantothenic, tannic, ascorbic and valeric acids.

Base salts include, but are not limited to, those formed with pharmaceutically acceptable cations, such as sodium, potassium, lithium, calcium, magnesium, ammonium and alkylammonium.

Basic nitrogen-containing groups may be quarternised with such agents as lower alkyl halide, such as methyl, ethyl, propyl, and butyl chlorides, bromides and iodides; dialkyl sulfates like dimethyl and diethyl sulfate; and others.

It will also be recognised that some compounds of formula (I) may possess asymmetric centres and are therefore capable of existing in more than one stereoisomeric form. The invention thus also relates to compounds in substantially pure isomeric form at one or more asymmetric centres eg., greater than about 90% ee, such as about 95% or 97% ee or greater than 99% ee, as well as mixtures, including racemic mixtures, thereof. Such isomers may be prepared by asymmetric synthesis, for example using chiral intermediates, or by chiral resolution.

In another aspect, the invention provides a method of treating, preventing or diagnosing a disease or condition wherein MIF cytokine or biological activity is implicated comprising the administration of a treatment, prevention or diagnostic effective amount of a compound of formula (I) or a pharmaceutically acceptable salt or prodrug thereof to a subject in need thereof.

In a further aspect, there is provided the use of a compound of formula (I) or a

pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment, prevention or diagnosis of a disease or condition wherein MIF cytokine or biological activity is implicated.

As used herein, MIF includes human or other animal MIF and derivatives and naturally occurring variants thereof which at least partially retain MIF cytokine or biological activity. Thus, the subject to be treated may be human or other animal such as a mammal. Non-human subjects include, but are not limited to primates, livestock animals (eg sheep, cows, horses, pigs, goats), domestic animals (eg dogs, cats), birds and laboratory test animals (eg mice rats, guinea pigs, rabbits). MIF is also expressed in plants (thus "MIF" may also refer to plant MIF) and where appropriate, compounds of Formula (I) may be used in botanical/agricultural applications such as crop control.

Reference herein to "cytokine or biological activity" of MIF includes the cytokine or biological effect on cellular function via autocrine, endocrine, paracrine, cytokine, hormone or growth factor activity or via intracellular effects.

In a further aspect of the invention there is provided a method of treating, diagnosing, or preventing autoimmune diseases, haemopoitic tumours or chronic or acute inflammatory diseases comprising administration of a treatment, diagnosis or prevention effective amount of a compound of formula (I), or a pharmaceutically acceptable salt or prodrug thereof. Such diseases include:

Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to

diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiostatis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg Alzheimers, multiple sclerosis), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing,

A further aspect of the invention provides for the use of a compound of Formula (I) or a pharmaceutically acceptable salt or prodrug thereof in the manufacture of a medicament for the treatment of a disease or condition as above.

As used herein, the term "effective amount" relates to an amount of compound which, when administered according to a desired dosing regimen, provides the desired MIF cytokine inhibiting or treatment or therapeutic activity, or disease/condition prevention. Dosing may occur at intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods. A cytokine or biological activity inhibiting amount is an amount which will at least partially inhibit the cytokine or biological activity of MIF. A therapeutic, or treatment, effective amount is an amount of the compound which, when administered according to a desired dosing regimen, is sufficient to at least partially attain the desired therapeutic effect, or delay the onset of, or inhibit the progression of or halt or partially or fully reverse the onset or progression of a particular

disease condition being treated. A prevention effective amount is an amount of compound which when administered according to the desired dosing regimen is sufficient to at least partially prevent or delay the onset of a particular disease or condition. A diagnostic effective amount of compound is an amount sufficient to bind to MIF to enable detection of the MIF-compound complex such that diagnosis of a disease or condition is possible.

Suitable dosages may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 µg to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1µg to 1mg per kg of body weight per dosage.

Suitable dosage amounts and dosing regimens can be determined by the attending physician or veterinarian and may depend on the desired level of inhibiting activity, the particular condition being treated, the severity of the condition as well as the general age, health and weight of the subject.

The active ingredient may be administered in a single dose or a series of doses. While it is possible for the active ingredient to be administered alone, it is preferable to present it as a composition, preferably as a pharmaceutical composition.

In a further aspect of the invention, there is provided a pharmaceutical composition comprising a compound of formula (I) together with a pharmaceutically acceptable carrier, diluent or excipient.

The formulation of such compositions is well known to those skilled in the art. The composition may contain pharmaceutically acceptable additives such as carriers, diluents or excipients. These include, where appropriate, all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be

understood that the compositions of the invention may also include other supplementary physiologically active agents.

The carrier must be pharmaceutically acceptable in the sense of being compatible with the other ingredients of the composition and not injurious to the subject. Compositions include those suitable for oral, rectal, inhalational, nasal, transdermal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intraspinal, intravenous and intradermal) administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Depending on the disease or condition to be treated, it may or may not be desirable for a compound of Formula (I) to cross the blood/brain barrier. Thus the compositions for use in the present invention may be formulated to be water or lipid soluble.

Compositions of the present invention suitable for oral administration may be presented as discrete units such as capsules, sachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or a suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (eg inert diluent, preservative, disintegrant (eg. sodium starch glycolate, cross-linked polyvinyl pyrrolidone, cross-linked sodium carboxymethyl cellulose)) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to

provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating, to provide release in parts of the gut other than the stomach.

Compositions suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured base, usually sucrose and acacia or tragacanth gum; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia gum; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

The compounds of Formula (I) may also be administered intranasally or via inhalation, for example by atomiser, aerosol or nebulizer means.

Compositions suitable for topical administration to the skin may comprise the compounds dissolved or suspended in any suitable carrier or base and may be in the form of lotions, gel, creams, pastes, ointments and the like. Suitable carriers include mineral oil, propylene glycol, polyoxyethylene, polyoxypropylene, emulsifying wax, sorbitan monostearate, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water. Transdermal devices, such as patches, may also be used to administer the compounds of the invention.

Compositions for rectal administration may be presented as a suppository with a suitable carrier base comprising, for example, cocoa butter, gelatin, glycerin or polyethylene glycol.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bactericides and solutes which render the composition isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents

and thickening agents. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the active ingredient.

It should be understood that in addition to the active ingredients particularly mentioned above, the compositions of this invention may include other agents conventional in the art having regard to the type of composition in question, for example, those suitable for oral administration may include such further agents as binders, sweeteners, thickeners, flavouring agents, disintegrating agents, coating agents, preservatives, lubricants and/or time delay agents. Suitable sweeteners include sucrose, lactose, glucose, aspartame or saccharine. Suitable disintegrating agents include corn starch, methylcellulose, polyvinylpyrrolidone, xanthan gum, bentonite, alginic acid or agar. Suitable flavouring agents include peppermint oil, oil of wintergreen, cherry, orange or raspberry flavouring. Suitable coating agents include polymers or copolymers of acrylic acid and/or methacrylic acid and/or their esters, waxes, fatty alcohols, zein, shellac or gluten. Suitable preservatives include sodium benzoate, vitamin E, alpha-tocopherol, ascorbic acid, methyl paraben, propyl paraben or sodium bisulphite. Suitable lubricants include magnesium stearate, stearic acid, sodium oleate, sodium chloride or talc. Suitable time delay agents include glyceryl monostearate or glyceryl distearate.

It will be recognised that other therapeutically active agents such as anti-inflammatory (eg steroids such as glucocorticoids) or anti-cancer agents may be used in conjunction with a compound of Formula (I). Compounds of Formula (I) when administered in conjunction with other therapeutically active agents may exhibit an additive or synergistic effect. These may be administered simultaneously, either as a combined form (ie as a single composition containing the active agents) or as discrete dosages. Alternatively, the other therapeutically active agents may be administered sequentially or separately with the compounds of the invention. Thus, the invention also relates to kits and combinations,

comprising a compound of Formula (I) and one or more other therapeutically active ingredients for use in the treatment of diseases or conditions described herein. Without being limiting, examples of agents which could be used in combination with a compound of Formula (I) include: antirheumatic drugs (including but not limited to methotrexate, leflunomide, sulphasalazine, hydroxycholorquine, gold salts); immunosuppressive drugs (including but not limited to cyclosporin, mycophenyllate mofetil, azathioprine, cyclophosphamide); anti-cytokine therapies (including but not limited to antagonists of, antibodies to, binding proteins for, or soluble receptors for tumor necrosis factor, interleukin 1, interleukin 3, interleukin 5, interleukin 6, interleukin 8, interleukin 12, interleukin 18, interleukin 17, and other pro-inflammatory cytokines as may be found relevant to pathological states); antagonists or inhibitors of mitogen-activated protein (MAP) kinases (including but not limited to antagonists or inhibitors of extracellular signal-regulated kinases (ERK), the c-Jun N-terminal kinases/stress-activated protein kinases (JNK/SAPK), and the p38 MAP kinases, and other kinases or enzymes or proteins involved in MAP kinase-dependent cell activation); antagonists or inhibitors of the nuclear factor kappa-B (NF-κB) signal transduction pathway (including but not limited to antagonists or inhibitors of I-κB-kinase, interleukin receptor activated kinase, and other kinases or enzymes or proteins involved in NF-κB-dependent cell activation); antibodies, protein therapeutics, or small molecule therapeutics interacting with adhesion molecules and co-stimulatory molecules (including but not limited to therapeutic agents directed against intercellular adhesion molecule-1, CD40, CD40-ligand, CD28, CD4, CD-3, selectins such as P-selectin or E-selectin); bronchodilators such as β-adrenoceptor agonists or anti-cholinergics; antagonists of eicosanoid synthesis pathways such as non-steroidal anti-inflammatory drugs, cyclooxygenase-2 inhibitors, thromboxane inhibitors, or lipoxygenase inhibitors; antibodies or other agents directed against leukocyte surface antigens (including but not limited to antibodies or other agents directed against CD3, CD4, CD5, CD19, CD20, HLA molecules); agents used for the treatment of inflammatory bowel disease (including but not limited to sulphasalazine, mesalazine, salicylic acid derivatives); anti-cancer drugs (including but not limited to cytotoxic drugs, cytolytic drugs, monoclonal antibodies).

In another aspect, the invention provides a method of treating or preventing a disease or condition wherein MIF cytokine or biological activity is implicated comprising:

administering to a mammal a compound of formula (I) and a second therapeutic agent.

In a preferred embodiment of this aspect of the invention, the second therapeutic agent is a glucocorticoid compound.

In another aspect, the present invention provides a method of prophylaxis or treatment of a disease or condition for which treatment with a glucocorticoid is indicated, said method comprising:

administering to a mammal a glucocorticoid and a compound of formula (I).

In yet another aspect, the present invention provides a method of treating steroid-resistant diseases comprising:

administering to a mammal a glucocorticoid and a compound of formula (I).

In a further aspect, the present invention provides a method of enhancing the effect of a glucocorticoid in mammals comprising administering a compound of formula (I) simultaneously, separately or sequentially with said glucocorticoid.

In yet a further aspect, the present invention provides a composition comprising a glucocorticoid and a compound of formula (I).

In a further aspect of the invention there is provided a use of a glucocorticoid in the manufacture of a medicament for administration with a compound of formula (I) for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

In yet a further aspect of the invention there is provided a use of a compound of formula (I) in the manufacture of a medicament for administration with a glucocorticoid for the treatment or prophylaxis of a disease or condition for which treatment of a glucocorticoid is indicated.

In yet a further aspect of the invention there is provided a use of a glucocorticoid and a compound of formula (I) in the manufacture of a medicament for the treatment or prophylaxis of a disease or condition for which treatment with a glucocorticoid is indicated.

Preferably the amount of glucocorticoid used in the methods, uses and compositions of the invention is less than the amount which would be effective in the absence of the compound of formula (I). In the treatment of steroid-resistant diseases or conditions which are not responsive to glucocorticoids, any amount of glucocorticoid which is effective in combination with a compound of formula (I) is considered less than the amount which would be effective in the absence of a compound formula (I). Accordingly, the invention provides a steroid-sparing therapy.

In preferred embodiments of the invention, the glucocorticoid and the compound of formula (I) are used to treat or prevent a disease or condition in a mammal, preferably in a human subject.

The term "disease or condition for which treatment with a glucocorticoid is indicated" refers to diseases or conditions which are capable of being treated by administration of a glucocorticoid including but not limited to autoimmune diseases, solid or haemopoitic tumours, or chronic or acute inflammatory diseases. Examples of such diseases or conditions include:

Rheumatic diseases (including but not limited to rheumatoid arthritis, osteoarthritis, psoriatic arthritis) spondyloarthropathies (including but not limited to ankylosing spondylitis, reactive arthritis, Reiter's syndrome), crystal arthropathies (including but not limited to gout, pseudogout, calcium pyrophosphate deposition disease), Lyme disease, connective tissue diseases (including but not limited to systemic lupus erythematosus, systemic sclerosis, polymyositis, dermatomyositis, Sjögren's syndrome), vasculitides (including but not limited to polyarteritis nodosa, Wegener's granulomatosis, Churg-Strauss syndrome), glomerulonephritis, inflammatory bowel disease (including but not limited to ulcerative colitis, Crohn's disease), peptic ulceration, gastritis, oesophagitis, liver disease (including but not limited to cirrhosis, hepatitis), autoimmune diseases (including but not limited to

diabetes mellitus, thyroiditis, myasthenia gravis, sclerosing cholangitis, primary biliary cirrhosis), pulmonary diseases (including but not limited to diffuse interstitial lung diseases, pneumoconioses, fibrosing alveolitis, asthma, bronchitis, bronchiostatis, chronic obstructive pulmonary disease, adult respiratory distress syndrome), cancers whether primary or metastatic (including but not limited to colon cancer, lymphoma, lung cancer, melanoma, prostate cancer, breast cancer, stomach cancer, leukemia, cervical cancer and metastatic cancer), atherosclerosis (eg ischaemic heart disease, myocardial infarction, stroke, peripheral vascular disease), disorders of the hypothalamic-pituitary-adrenal axis, brain disorders (eg Alzheimers, multiple sclerosis), corneal disease, iritis, iridocyclitis, cataracts, uveitis, sarcoidosis, diseases characterised by modified angiogenesis (eg diabetic retinopathy, rheumatoid arthritis, cancer), endometrial function (menstruation, implantation, endometriosis), psoriasis, endotoxic (septic) shock, exotoxic (septic) shock, infective (true septic) shock, other complications of infection, pelvic inflammatory disease, transplant rejection, allergies, allergic rhinitis, bone diseases (eg osteoporosis, Paget's disease), atopic dermatitis, UV(B)-induced dermal cell activation (eg sunburn, skin cancer), malarial complications, diabetes mellitus, pain, inflammatory consequences of trauma or ischaemia, testicular dysfunctions and wound healing.

These diseases or conditions may also include steroid-resistant diseases or conditions where treatment with a glucocorticoid is indicated, but where the glucocorticoid is ineffective or is not as effective as expected.

The methods of the invention are preferably performed in a steroid-sparing manner. The term "steroid-sparing" refers to a combination therapy method that allows a reduction in the amount of glucocorticoid administered while still providing an effective therapy for the disease or condition being treated or prevented.

Steroid-resistant diseases or conditions are diseases or conditions for which treatment with a glucocorticoid is indicated, but where the glucocorticoid is ineffective or is not as effective as expected. This term encompasses diseases or conditions for which the effective dose of glucocorticoid results in unacceptable side effects and/or toxicity. Some steroid-resistant diseases or conditions may require a dosage of glucocorticoid so large that

they are considered non-responsive and therefore are not able to be successfully treated with glucocorticoids. Some steroid-resistant diseases or conditions may require a large dosage of glucocorticoid to achieve only a small effect on the symptoms of the disease or condition. Furthermore, some patients, diseases or conditions present with symptoms that do not respond to treatment with a glucocorticoid, or may become less sensitive to glucocorticoid treatment over time.

Glucocorticoids are a group of steroid hormones, which are used to treat or prevent a wide range of diseases or conditions. Suitable glucocorticoids may be synthetic or naturally occurring and include but are not limited to prednisolone, prednisone, cortisone acetate, beclamethasone, fluticasone, hydrocortisone, dexamethasone, methyl prednisolone, triamcinolone, budesonide and betamethasone.

In preferred embodiments of the invention, the glucocorticoid used is selected from prednisone, prednisolone, hydrocortisone, fluticasone, beclamethasone, betamethasone, methyl prednisolone, budesonide, triamcinolone, dexamethasone and cortisone. Most preferably, the glucocorticoid is selected from prednisone, prednisolone, methyl prednisolone, fluticasone and beclamethasone. Beclamethasone and fluticasone are particularly preferred for treating asthma. Prednisone, prednisolone and methyl prednisolone are particularly preferred in the treatment of systemic or local inflammatory diseases.

The amounts of glucocorticoid and compound of formula (I) are selected such that in combination they provide complete or partial treatment or prophylaxis of a disease or condition for which a glucocorticoid is indicated. The amount of compound formula (I) is preferably an amount that will at least partially inhibit the cytokine or biological activity of MIF. The amount of glucocorticoid is preferably less than the amount required in the absence of the compound of formula (I). The amounts of glucocorticoid and compound of formula (I) used in a treatment or therapy are selected such that in combination they at least partially attain the desired therapeutic effect, or delay onset of, or inhibit the progression of, or halt or partially or fully reverse the onset or progression of the disease or condition being treated. The amounts of glucocorticoid and compound of formula (I) used in the prophylaxis of a disease or condition are selected such that in combination they at least partially prevent or delay the onset of the disease or condition. Dosing may occur at

intervals of minutes, hours, days, weeks, months or years or continuously over any one of these periods.

Suitable doses of a compound of formula (I) may lie within the range of about 0.1 ng per kg of body weight to 1 g per kg of body weight per dosage. The dosage is preferably in the range of 1 µg to 1 g per kg of body weight per dosage, such as is in the range of 1 mg to 1 g per kg of body weight per dosage. In one embodiment, the dosage is in the range of 1 mg to 500 mg per kg of body weight per dosage. In another embodiment, the dosage is in the range of 1 mg to 250 mg per kg of body weight per dosage. In yet another preferred embodiment, the dosage is in the range of 1 mg to 100 mg per kg of body weight per dosage, such as up to 50 mg per kg of body weight per dosage. In yet another embodiment, the dosage is in the range of 1µg to 1mg per kg of body weight per dosage.

Suitable dosage amounts of glucocorticoids will depend, in part, on the mode of administration and whether the dosage is being administered in a single, daily or divided dose, or as a continuous infusion. When administered orally, intravenously, intramuscularly, intralesionally or intracavity (eg. intra-articular, intrathecal, intrathoracic), dosages are typically between 1 mg to 1000 mg, preferably 1 mg to 100 mg, more preferably 1 mg to 50 mg or 1 mg to 10 mg per dose. When administered topically or by inhalation as a single, daily or divided dose, dosages are typically 1 ng to 1 µg, 1 ng to 1 mg or 1 pg to 1 µg.

Suitable dosage amounts and dosing regimens can be determined by the attending physician or veterinarian and may depend on the desired level of inhibiting activity, the particular condition being treated, the severity of the condition as well as the general age, health and weight of the subject.

The glucocorticoid and compound of formula (I) may be administered simultaneously or sequentially. The active ingredients may be administered alone but are preferably administered as a pharmaceutically acceptable composition or separate pharmaceutically acceptable compositions.

The formulation of such compositions is well known to those skilled in the art and are described above in relation to compounds of formula (I). The composition or

compositions may contain pharmaceutically acceptable additives such as carriers, diluents or excipients. These include, where appropriate, all conventional solvents, dispersion agents, fillers, solid carriers, coating agents, antifungal and antibacterial agents, dermal penetration agents, surfactants, isotonic and absorption agents and the like. It will be understood that the compositions of the invention may also include other supplementary physiologically active agents.

Preferred unit dosage compositions are those containing a daily dose or unit, daily sub-dose, as herein above described, or an appropriate fraction thereof, of the glucocorticoids and/or compound of formula (I) which inihibit the cytokine or biological activity of MIF.

The compounds of formula (I), either as the only active agent or together with another active agent, eg: a glucocorticoid, may also be presented for use in veterinary compositions. These may be prepared by any suitable means known in the art. Examples of such compositions include those adapted for:

(a) oral administration, external application (eg drenches including aqueous and non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pellets for admixture with feedstuffs, pastes for application to the tongue;

(b) parenteral administration, eg subcutaneous, intramuscular or intravenous injection as a sterile solution or suspension; and

(c) topical application eg creams, ointments, gels, lotions, etc.

By virtue of their ability to bind to or antagonize MIF, compounds of Formula (I) or salts or derivatives thereof may be used as laboratory or diagnostic or *in vivo* imaging reagents. Typically, for such use the compounds would be labelled in some way, for example, radio isotope, fluorescence or colorimetric labelling, or be chelator conjugated. In particular, compounds of Formula (I) could be used as part of an assay system for MIF or as controls in screens for identifying other inhibitors. Those skilled in the art are familiar with such screens and could readily establish such screens using compounds of Formula (I). Those skilled in the art will also be familiar with the use of chelate conjugated molecules for *in vivo* diagnostic imaging.

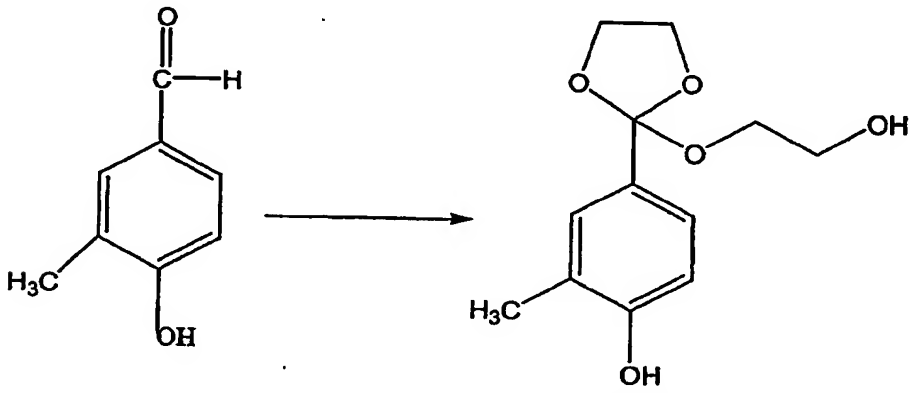
Unless the context indicates otherwise, reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in Australia.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

The invention will now be described with reference to the following examples which are included for the purpose of illustration only and are not intended to limit the generality of the invention hereinbefore described.

EXAMPLES

Example 1: Preparation of 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1)



Compound 1

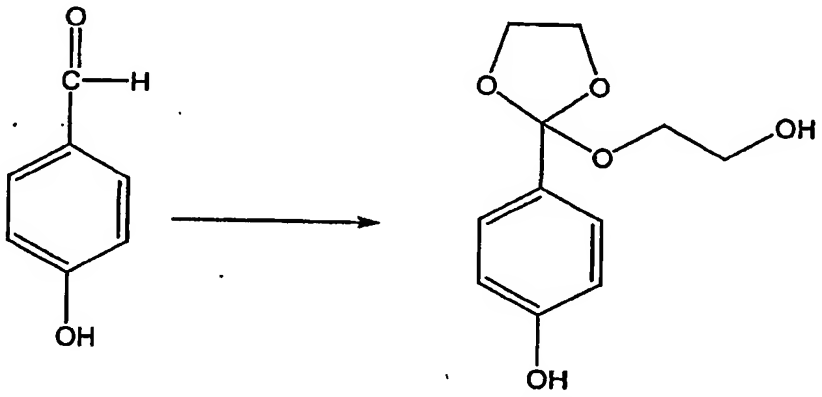
A mixture of 3-methyl-*p*-hydroxybenzaldehyde (0.5 g, 3.6 mmol), ethylene glycol (0.34 g, 5.5 mmol) and *p*-sulfonic acid (0.07 g, 0.36 mmol) in toluene was heated under reflux. After 24 h, the reaction mixture was cooled to room temperature, TLC showed no starting material. The toluene was removed *in vacuo* and saturated solution of sodium hydrogen

carbonate (20 ml) was added to the residue, which was then extracted with ethyl acetate (3 x 20 ml). The organic layer was washed with water (20 ml), dried over anhydrous sodium sulfate and the solvent was removed *in vacuo*. The residue was then recrystallised from an ethyl acetate and hexane mixture to give the product as a brown solid in 24% yield.

^{1}H NMR ($CDCl_3$): 2.21 (s, 3H), 3.62 (t, 2H, J = 4.5 Hz), 3.70 (t, 2H, J = 4.2 Hz), 3.81 (t, 2H, J = 4.7 Hz), 4.42 (t, 2H, J = 4.7 Hz), 6.74 (d, 1H, J = 8.4 Hz), 7.21 (d, 1H, J = 8.4 Hz) and 7.78 (s, 1H). MS: m/e 263 (M^+ + Na), 179 (M^+ - OCH_2CH_2OH), 147, 135, 118 and 107.

^{13}C NMR ($CDCl_3$): 15.7, 61.1, 63.5, 69.1, 72.4, 114.0, 120.5, 124.5, 128.4, 135.6, 159.8 and 167.1.

Example 2: Preparation of 2-(2-hydroxyethoxy)-2-(4-hydroxyphenyl)-1,3-dioxolane (Compound 2)



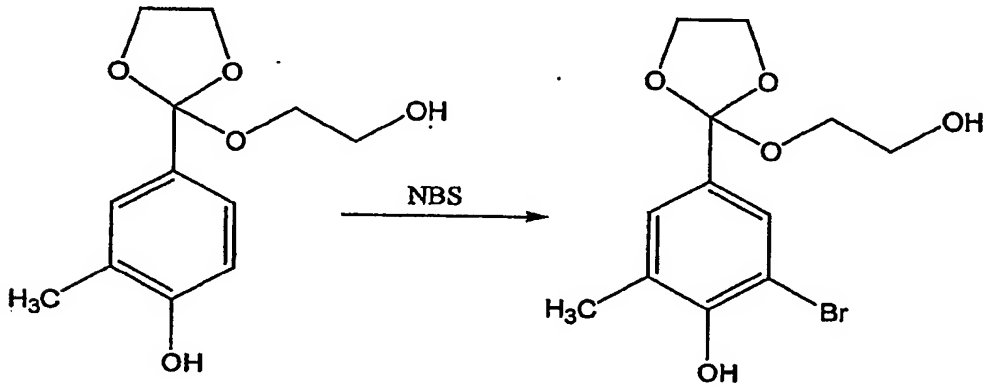
Compound 2

To a solution of *p*-hydroxybenzaldehyde (1g, 8.18 mmol) in anhydrous toluene (100 mL) was added ethylene glycol (0.68 mL, 12.28 mmol), pyridinium toluene sulphonate (0.2 g, 0.88 mmol) and *p*-toluene sulphonic acid monohydrate (0.16 g, 0.88 mmol). The solution was refluxed overnight before concentrating the solvent to furnish an amber gum. Saturated sodium bicarbonate (50 mL) was then added to the reaction mixture, and extracted with ethyl acetate (3x 50 mL). The organic extracts were dried over magnesium sulphate, filtered, and concentrated to furnish a dark brown gum. The gum was

chromatographed on silica (ether/methanol, 9.5:0.5) to furnish the title compound as a dark brown solid (173 mg, 9%).

^{1}H NMR ($CDCl_3$): δ 7.88 (d, 2H, 2x ArCH, *J* 8.7 Hz), 7.49 (bs, 1H, phenolic hydroxyl), 6.82 (d, 2H, 2x ArCH, *J* 8.7 Hz), 4.46, 3.84 (2x appt, 2x 2H, 2x ethoxy CH_2, J_{vic} 4.5 Hz), 3.74 (m, 2H, dioxolan CH_2), 3.66 (appt, 2H, dioxolan CH_2, J_{vic} 4.8 Hz);
LRMS (ESI): *m/z* 227 [M+H$^+$]; $C_{11}H_{14}O_5$: 226.23

Example 3: Preparation of 2-(2-hydroxyethoxy)-2-(3-bromo-4-hydroxy-5-methylphenyl)-1,3-dioxolane (compound 3)



Compound 3

A mixture of compound 1 (109 mg; 0.4 mmol), *N*-bromosuccinamide (80 mg; 0.4 mmol) and AIBN (7.3 mg; 0.045 mmol) in dry carbon tetrachloride was refluxed for 5 hrs, TLC did not show any of the starting material. The reaction mixture was cooled to room temperature and then concentrated to dryness. The residue was dissolved in ethyl acetate (2 x 10 mls). The clear solution was washed with distilled water (5 x 10 ml), dried over anhydrous Na_2SO_4 and evaporated to dryness. This gave 47% yield.

^{1}H NMR ($CDCl_3$): 2.32 (s, 3H), 3.65 (t, 2H, J = 4.4 Hz), 3.74 (t, 2H, J = 4.3 Hz), 3.83 (t, 2H, J = 4.7 Hz), 4.46 (t, 2H, J = 4.8 Hz), 7.80 (s, 1H) and 8.02 (s, 1H).

BIOLOGICAL EXAMPLES

Biological Example 1: MIF-induced fibroblast proliferation.

Methods

The activity of a compound of formula (I) was studied in a bioassay utilising MIF-induced proliferation of human dermal fibroblasts. The proliferation of human fibroblasts has been demonstrated to be a phenomenon inducible by MIF (16). S112 human dermal fibroblasts were propagated in RPMI/10% foetal calf serum (FCS). Prior to experimentation, cells were seeded at 10^5 cells/ml in RPMI/0.1% BSA for 18 hours. Cells were treated with recombinant human macrophage migration inhibitory factor (MIF) 50 ng/ml and/or a compound of the invention at a concentration of 1 nM. The compound was combined with MIF at time point –30 minutes, prior to adding to cell culture at time point zero. At time point zero, culture medium was replaced with RPMI/10% FCS and treatments administered. At time point 30 hours, cells were pulsed with 1 μCi ^{3}H-thymidine. At time point 48 hours, cells were harvested using a semi-automated cell harvester. The radioactivity incorporated into DNA was determined by liquid scintillation counting, with results expressed as [^{3}H] thymidine incorporation.

Significant inhibition of MIF-induced proliferation was determined by the demonstration of a significant P value ($P < 0.05$) using the Mann-Whitney U-test.

Results

2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1), when used in the method above, significantly inhibited the induction of S112 human fibroblast proliferation ($P < 0.05$), as shown in Table 1 and Figure 1. Treatment of cells with MIF (+MIF) induced proliferation, but this was prevented by pre-incubating MIF with Compound 1 (1 nM) (+MIF +cpd 1) (*$P < 0.05$).

Table 1.

	Untreated cells	MIF-treated cells	MIF-treated cells + cpd1 at 1 nM
Mean (cpm)	3245	4415	2994 *
Standard error	393.1	403.5	410.7
Number of experiments	9	9	9

* $P < 0.05$

Biological Example 2: MIF-dependent IL-1 induced fibroblast cyclooxygenase-2 expression.

Methods

The activity of the compounds of formula (I) were further studied in a bioassay utilising MIF-dependent activation of human dermal fibroblasts. Sampey *et al* have shown that induction of the expression of cyclooxygenase-2 (COX-2) by the cytokine interleukin 1 (IL-1) is dependent upon the presence of MIF, i.e. can be prevented using specific anti-MIF monoclonal antibody (17). IL-1-induced COX-2 expression is therefore a MIF-dependent event.

S112 human dermal fibroblasts were propagated in RPMI/10% foetal calf serum (FCS). Prior to experimentation, cells were seeded at 10^5 cells/ml in RPMI/0.1% BSA for 18 hours. Cells were treated with recombinant human IL-1 (0.1 ng/ml) and with compound at 1-100 μM. After 6 hours, cells were collected and intracellular COX-2 protein determined by permeabilisation flow cytometry, as described by Sampey *et al* (18). Cells permeabilised with 0.1% saponin were sequentially labelled with a mouse anti-human COX-2 monoclonal antibody and with sheep-anti-mouse F(ab)2 fragment labelled with fluoroscein isothiocyanate. Cellular fluorescence was determined using a flow cytometer. At least 5000 events were counted for each reading, each of which was performed in duplicate, and the results expressed in mean fluorescence intensity (MFI) after subtraction of negative control-labelled cell fluorescence.

In Table 2 and Figure 2, the effect of each concentration of 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1) was determined by subtracting the IL-1+compound-treated cell MFI from the IL-1-treated cell MFI, and expressed as % inhibition. Significant inhibition of IL-induced COX-2 expression was determined by the demonstration of a significant P value ($P < 0.05$) using Student's test.

Results

As shown in Figure 2, cells treated with Compound 1 exhibited a significant reduction in COX-2 expression as measured by flow cytometry ($P < 0.01$). Statistically significant inhibition of the induction of COX-2 expression by IL-1 in human S112 fibroblast cells was demonstrated when cells were treated with Compound 1 (cpd 1) 50 μM (*$P < 0.01$).

As shown in Table 2 and Figure 3, cells treated with 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1) exhibited a dose-dependent reduction in COX-2 expression as measured by flow cytometry.

Table 2.

Concentration of Compound 1 (μM)	Mean % inhibition COX2 expression	Standard error	Number of experiments
0.01	10.5	9.6	4
0.1	13.2	9.5	4
1	15.6*	8.1	4
10	19.5*	3.9	6
50	31.4*	10.8	8

* $P < 0.05$

In Figure 4, the effect of 2-(2-hydroxyethoxy)-2-(4-hydroxyphenyl)-1,3-dioxolane (Compound 2) is expressed as mean fluorescence intensity (MFI), after deducting the MFI of control-labelled cells. Significant inhibition of IL-induced COX-2 expression was determined by the demonstration of a significant P value ($P < 0.05$) using Student's test. Significant inhibition of IL-induced COX-2 expression in human S112 fibroblast cells was demonstrated in cells treated with Compound 2 (IL-1+cpd2) compared to cells treated with IL-1 ($P < 0.05$).

Biological Example 3: MIF-dependent antigen-specific T cell activation.

Methods

The activity of compounds of formula (I) was further studied in a bioassay utilising MIF-dependent activation of murine T cell activation. The activation of T lymphocytes in response to exposure to a recall antigen is known to be dependent on the presence of MIF, i.e. can be prevented using specific anti-MIF monoclonal antibody, (7). Antigen-induced T cell activation is therefore a MIF-dependent phenomenon.

Splenocytes were obtained by saline flushing of spleens obtained from C57Bl/6 mice previously immunized with methylated bovine serum albumin (mBSA, Sigma Chemical Co., Castle Hill, Australia). Mice were immunized on day 0 with 200 μg mBSA emulsified in 0.2 ml of Freund's complete adjuvant (FCA) and injected subcutaneously into the flank skin. On day 7, the mice received 100μg mBSA/0.1 ml FCA by intradermal injection at the base of the tail. Spleens were removed on day 28 after first immunisation and a single cell suspension was prepared in DMEM containing 5% FCS and 0.05% 2-mercaptoethanol. 1×10^5 cells /200μl were cultured in triplicate in the presence of mBSA (10 μg /ml) with or without the addition of 2-(2-hydroxyethoxy)-2-(4-hydroxyphenyl)-1,3-dioxolane (Compound 2) at a concentration of 100 nM – 10 μM. The T cell proliferation response was determined by measuring the amount of [^{3}H] thymidine incorporation during the final 18 hr. The cells were harvested and radioactivity incorporation into the DNA was measured with a Wallac 1409 liquid scintillation counter (Pharmacia, Turku, Finland). Significant inhibition of T cell activation was determined by the demonstration of a significant P value ($P < 0.05$) using Student's test.

Results.

Treatment of spleen cells with 2-(2-hydroxyethoxy)-2-(4-hydroxyphenyl)-1,3-dioxolane (Compound 2) (cpd2) resulted in a significant dose-dependent reduction in antigen-specific T cell activation, compared to cells exposed to mBSA without Compound 2 ($P < 0.05$) (Figure 5).

Biological Example 4: Combination of MIF-antagonist with glucocorticoid: effects on MIF-dependent IL-1 induced fibroblast cyclooxygenase-2 expression.

A particular aspect of the biological function of MIF relates to its ability to antagonise the anti-inflammatory effects of glucocorticoids such as dexamethasone, as recently reviewed by Morand *et al.* (4). This property of MIF suggests that MIF antagonists might exert "steroid-sparing" effects, that is, their use in combination with glucocorticoids might permit the achievement of a greater therapeutic effect with a given dose of glucocorticoids. Thus, in the presence of MIF antagonists, low doses of glucocorticoids could exert a therapeutic effect otherwise requiring a higher dose of glucocorticoids. As the adverse effects of glucocorticoids are in general dose-dependent, the ability to reduce the requirement for glucocorticoids is clinically desirable.

The potential for a MIF antagonist to be "steroid-sparing", therefore, could be demonstrated the observation of enhanced effectiveness of a given dose of glucocorticoids in the presence of a MIF antagonist.

Methods

The above *in vitro* assay (Biological Example 2) for analysing the effect of MIF antagonists on IL-1 induced COX-2 expression was performed using 2-(2-hydroxyethoxy)-2-(4'-hydroxy-3'-methylphenyl)-1,3-dioxolane (Compound 1) (50 μM), dexamethasone (1 nM) or a combination of dexamethasone (1 nM) and Compound 1 (50 μM). COX-2 expression was expressed as the mean fluorescence intensity (MFI) as measured by flow cytometry, after deduction of the MFI for control-labelled samples, as described by Sampey *et al.* (18). The results are shown in Table 3 and Figure 6.

Results

Significant enhancement of the inhibitory effects of the glucocorticoid dexamethasone was determined by the demonstration of a significant P value ($P < 0.05$) using Student's test, compared to the effect of dexamethasone alone. Compared to the inhibition of IL-1-induced COX-2 expression achieved with 1 nM dexamethasone alone (IL-1+DEX), a significantly greater inhibition of IL-1-induced COX-2 expression was observed when

cells were treated with 1 nM dexamethasone together with Compound 1 50 μM (IL-1+DEX+cpd1) ($P < 0.05$).

Table 3.

	control	IL-1	IL-1 + DEX	IL-1+DEX+cpd1
Mean COX-2 expression (MFI)	0.7400	49.37	15.13	6.013*
Standard error	0.4413	2.412	1.770	2.906
n	4	4	4	4

* $P < 0.05$

Biological Example 5: Lack of cytotoxicity.

A valuable characteristic of a therapeutic material is a lack of toxicity. The compounds of formula (I) may have low toxicity towards cells. To examine this in vitro, the ability of 2-(2-hydroxyethoxy)-2-(4-hydroxy-3-methylphenyl)-1,3-dioxolane (Compound 1) to induce apoptosis ("programmed cell death) was investigated. A lack of cytotoxicity would be evidenced by the finding of equivalent proportions of apoptotic and viable cells in control- and compound-treated cells.

Methods

To examine the cytotoxicity of compounds of formula (I), S112 human dermal fibroblasts were exposed to a therapeutic concentration (50 μM) of Compound 1 or vehicle (control) and analysed apoptosis by flow cytometric analysis of annexin V and propidium iodide staining, as described by Leech *et al.* (19). Toxicity was assessed by analysis of apoptosis using flow cytometric detection of cell surface Annexin V binding and propidium iodide staining. At least 5000 events were analysed for each experiment. Cells positive for both Annexin V and propidium iodide were designated as apoptotic and cells negative for both Annexin V and propidium iodide were designated as viable. Results are expressed as the percentage (%) of cells with each of these labels.

Results.

The results of cytotoxicity analysis are shown in Figure 7. No significant increase in apoptotic cell numbers, and no significant decrease in viable cell numbers, was observed in cells treated with Compound 1 compared to control-treated cells.

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Figure 1

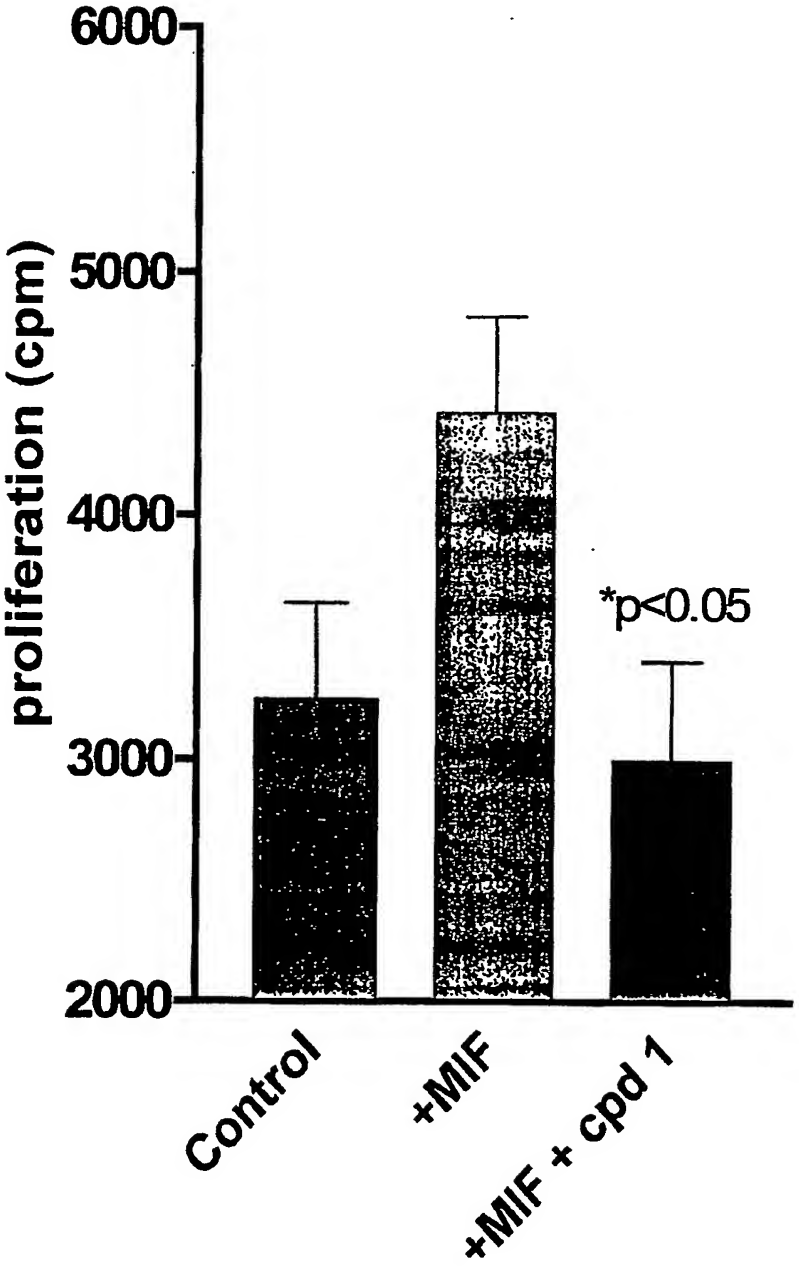

6000
5000
4000
3000
2000
proliferation (cpm)
*p<0.05
Control
+MIF
+MIF + cpd 1

Figure 2

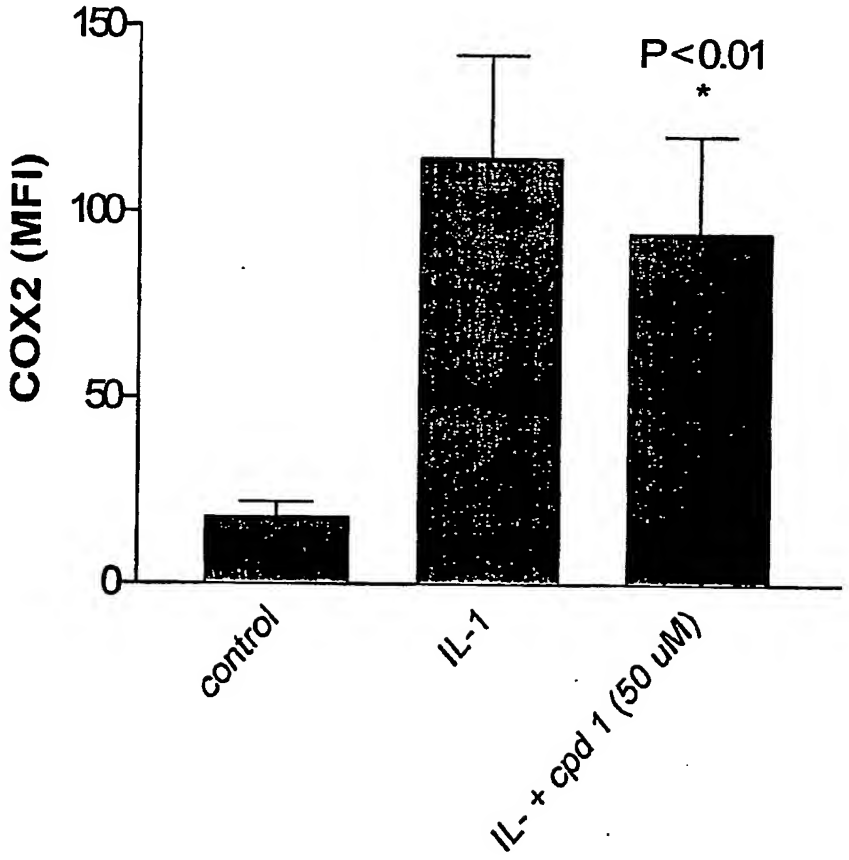


Figure 3

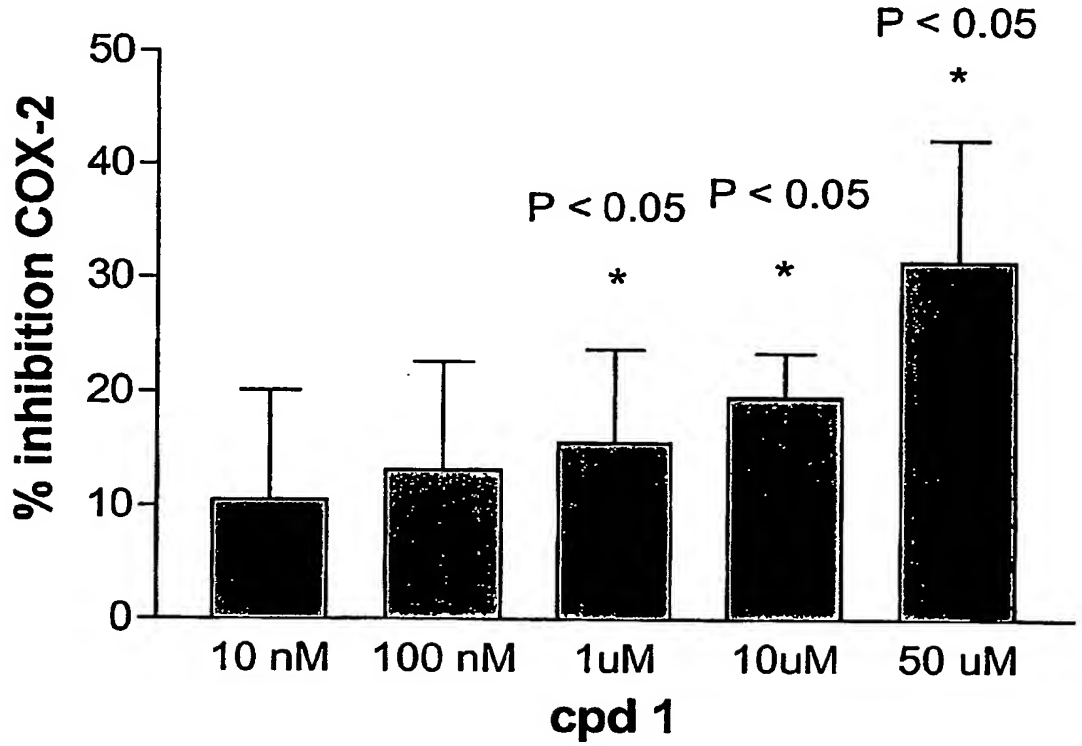

P < 0.05
*
P < 0.05
*
P < 0.05
*
% inhibition COX-2
50
40
30
20
10
0
10 nM
100 nM
1uM
10uM
50 uM
cpd 1

Figure 4

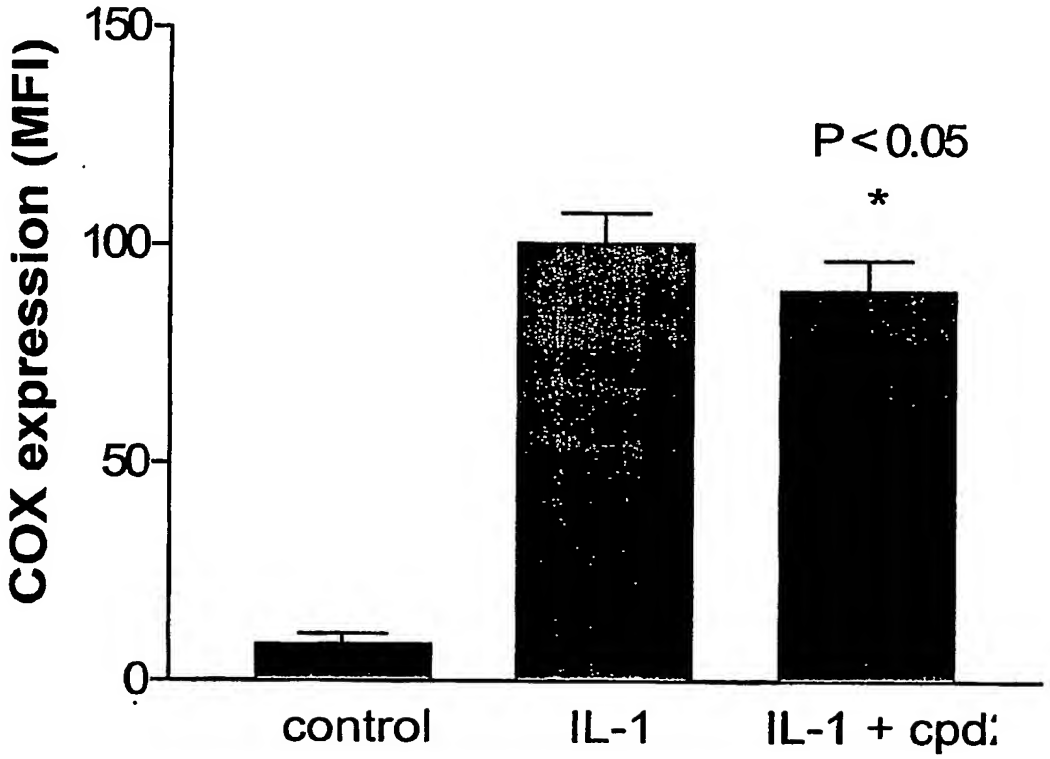


Figure 5

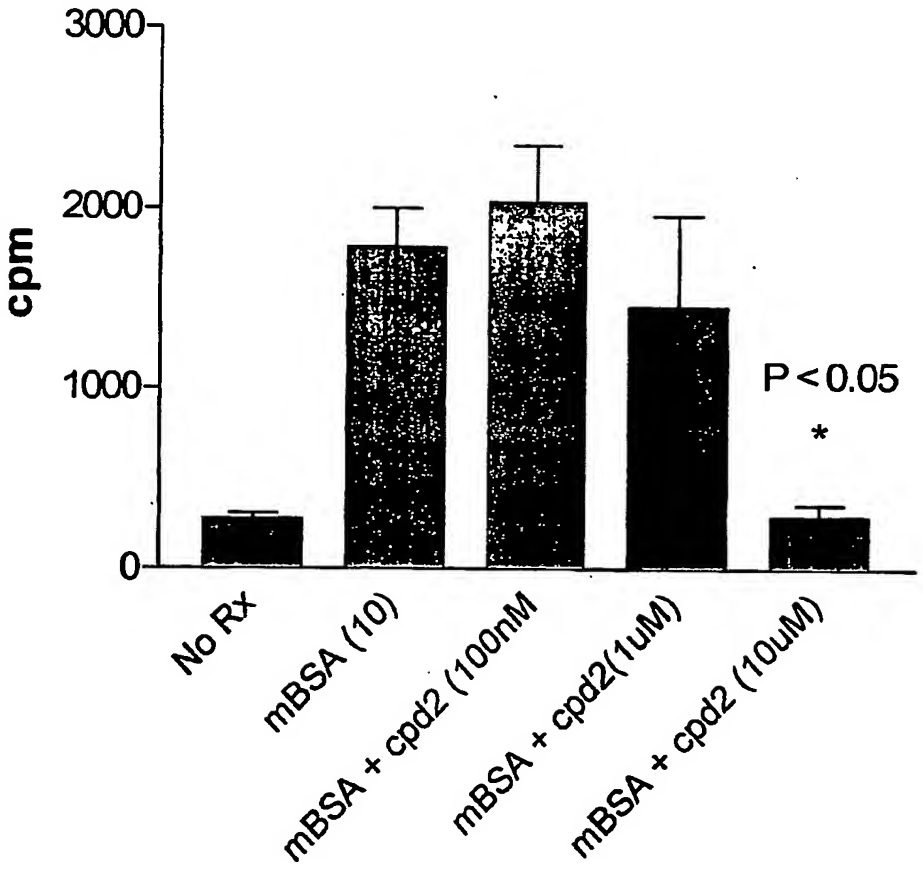

3000
2000
1000
0
cpm
P < 0.05
*
No Rx
mBSA (10)
mBSA + cpd2 (100nM
mBSA + cpd2(1uM)
mBSA + cpd2 (10uM)

Figure 6

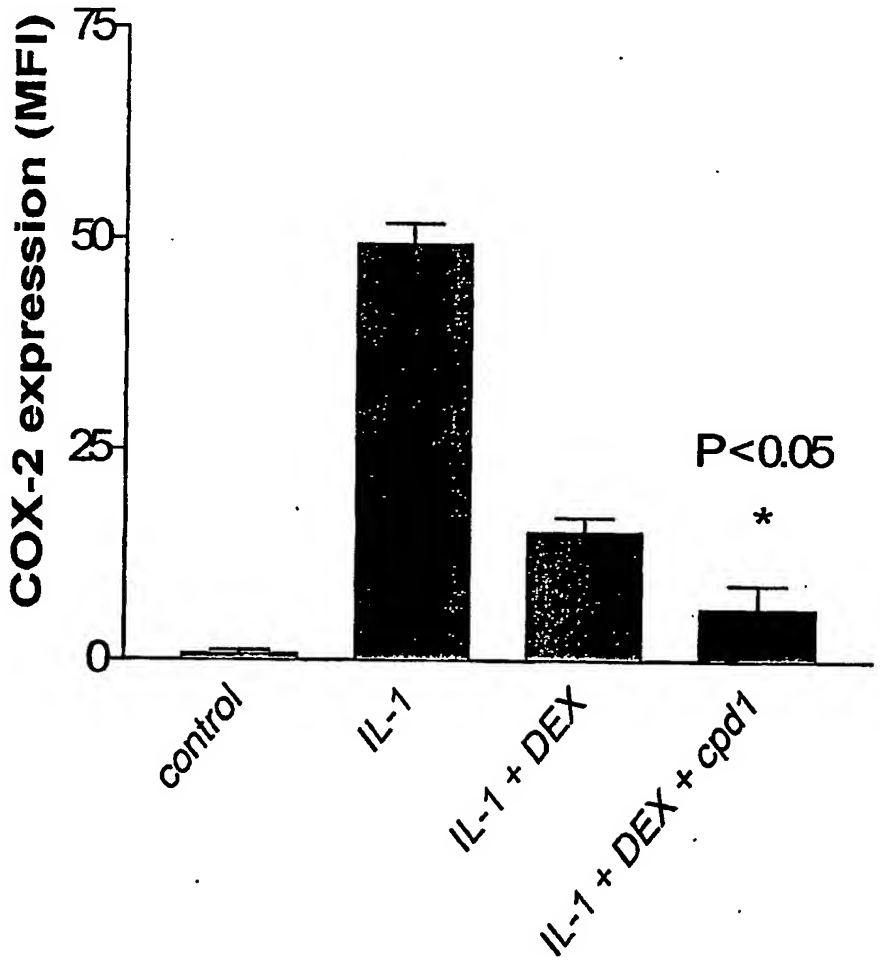
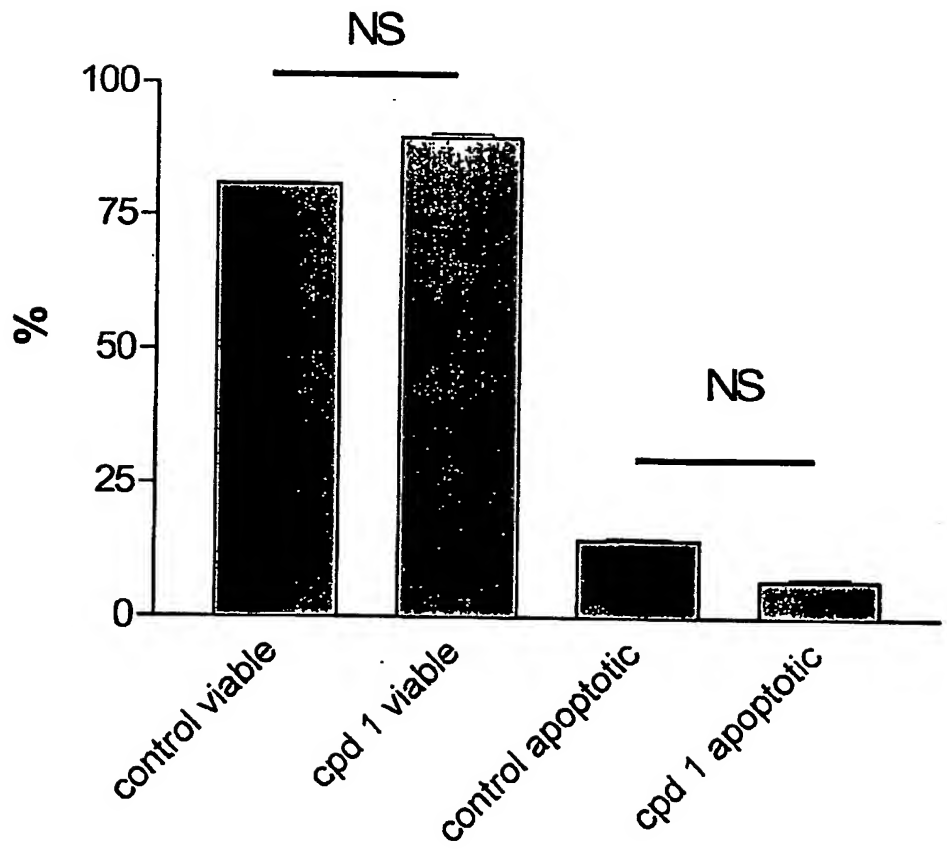

COX-2 expression (MFI)
75
50
25
0
P<0.05
*
control
IL-1
IL-1 + DEX
IL-1 + DEX + cpd1

Figure 7


NS
NS
100
75
50
25
0
%
control viable
cpd 1 viable
control apoptotic
cpd 1 apoptotic